A Cerebellum-like Circuit in the Auditory System Cancels Self-Generated Sounds

Shobhit Singla

Submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
under the Executive Committee
of the Graduate School of Arts and Sciences

Columbia University

2017

ABSTRACT

A Cerebellum-like Circuit in the Auditory System Cancels Self-Generated Sounds

Shobhit Singla

The first stage of mammalian auditory processing occurs within the dorsal and ventral divisions of the cochlear nucleus. The dorsal cochlear nucleus (DCN) is remarkable in that it shares striking similarities with the cerebellum in terms of its development, gene expression patterns, and anatomical organization. Notably, principal cells of the DCN integrate auditory nerve input with a diverse array of signals conveyed by a mossy fiber-granule cell system. Yet how the elaborate cerebellum-like circuitry of DCN contributes to early auditory processing has been a longstanding puzzle. The work in this thesis shows that, in mice, that the DCN functions to cancel responses to self-generated sounds. While the DCN and ventral cochlear nucleus (VCN) neurons respond similarly to externally-generated acoustic stimuli, sounds generated by licking behavior evoke much weaker responses in DCN than in VCN. Recordings in deafened mice revealed non-auditory signals related to licking in Purkinje-like neurons of DCN. Moreover, silencing somatosensory mossy fiber inputs revealed prominent DCN responses to sounds generated by licking, suggesting that these inputs normally function to cancel responses to self-generated sounds. Finally, I show that this cancellation is not fixed, but involves an adaptive process whereby neural responses correlated with the animal's own behavior are gradually reduced. Together, these findings suggest that the fundamental process of distinguishing self-generated from external stimuli begins at the very first stage of

mammalian auditory processing. Related adaptive filtering functions have been described for cerebellum-like sensory structures in fish and hypothesized for the mammalian cerebellum. Hence our findings also suggest that, despite their wide phylogenetic separation, different cerebellum-like structures and the cerebellum itself may all perform a similar computation.

Table of contents

List of Figures

Acknowledgements

This work would not have been possible without the support and guidance of a number of people.

I would like to first and foremost thank my advisor, Nate Sawtell. Because of his constant encouragement and advice, I am a better scientific thinker, writer, and communicator. Second, I'd like to thank the current and former members of the Sawtell Lab. Conor Dempsey helped with making the mimic of the lick sound and much of the analysis presented in this thesis. I spent many years in the lab with Tim Requarth, Karina Scalise, and Armen Enikolopov and more recently Kathryn Birkenbach. Their collaboration, discussions, and friendships fostered a laboratory culture that was a pleasure to work in over the past five years. Newer members Richard Warren, Anna Snyder, Abbey Zadina have only added to this positive working environement. Former lab managers Anna Safartay and Alyson Lowell, and current lab manager Rachel Rampil, made sure everything ran smoothly. Members of Bruno lab, Losconzy lab and Zuker lab made for an open collaborative environment on Hammer 5th floor as a whole.

I'd like to thank the members of my thesis committee: Charles Zuker, Larry Abbott, and Sarah Woolley for their feedback over the course of this Ph.D. I'd also like to thank Laurence Trussell for coming all the way from Oregon to serve as the external examiner.

I am grateful to the program directors of the Neurobiology and Behavior program, Darcy Kelley, Carol Mason and Ken Miller, and the program administrators, Alla Kerzhner and Rozanna Yakub. I would also like to thank the directors of the MD/PhD program, Michael Shelanski, Ron Liem, Patrice Spitalnik and Steve Reiner, as well as our program administrator, Jeffrey Brandt.

I would like to thank the many friends I have made throughout the Columbia neuroscience community in particular my fellow graduate students in lab, Tim Machado, Mariano Gabitto, and Amy Norovich. I would like to especially thank my girlfriend Virginia Workman whose love and support were instrumental throughout this process.

Finally I would like to thank my parents, brother, and sister-in-law. There is not enough space to describe all that they have done throughout the years. I am who I am because of them.

1. CHAPTER 1: INTRODUCTION

Cochlear output via the auditory nerve is processed in parallel at the very first stage of central auditory processing in two divisions of the cochlear nucleus: the dorsal and ventral divisions. The early neuroanatomist, Lorente de Nó, described the dorsal cochlear nucleus (DCN) as the "cerebellum of the auditory system," (de Nó 1979). Since then, why a neural circuit resembling the cerebellum exists at one of the first stages of auditory processing has remained a mystery (Oertel & Young 2004). The work in this thesis attempts to answer this question by using insights gained in other, remarkably similar, cerebellum-like structures found in electrosensory systems of fish. Studies in these systems showed that cerebellum-like sensory structures are able to cancel the effects of predictable, self-generated electrosensory input (Bell 1981; Bastian 1995; Bodznick & MONTGOMERY 1992). In Chapter 2, I use an awake, behaving mouse preparation to study responses in the ventral cochlear nucleus (VCN) and DCN to both externally and self-generated sounds. I will show that while the VCN, which lacks cerebellum-like circuitry, responds to both externally and self-generated sounds, the DCN responds preferentially to externally generated sounds while cancelling responses to self-generated sounds. In Chapter 3, I will show that prominent responses to self-generated sounds are revealed in DCN after silencing somatosensory mossy fibers. Finally, in Chapter 4, I will show how suppression found in Chapter 2 is not static but involves an adaptive process whereby neural responses correlated with the animal's own behavior are

gradually reduced. The ability to cancel self-generated sounds would be important to better perceive and react to behaviorally relevant, external, sounds.

1.1. **The Mammalian Auditory Pathway**

The ability to hear, or perceive sounds, begins when vibrations through the air or other medium are collected by the external ear (pinna) and are mechanically amplified by the tympanic membrane and the three small bones that make up the middle ear: the malleus, incus, and stapes. The sound wave is transmitted to the cochlea through the oval window via the foot of the stapes where it causes the basilar membrane to vibrate. Since the basilar membrane is stiffer at the base and more flexible at the apex, higher frequencies cause vibrations at the base while lower frequencies cause vibrations at the apex. Inner hair cells along the Organ of Corti, which lines the basilar membrane, transduce these mechanical vibrations to electrical signals that are eventually carried along the auditory nerve. Inner hair cells, and therefore auditory nerve fibers, are tuned to specific frequencies (characteristic or best frequency), which is a function of their location along the basilar membrane. As a result, functionally, the cochlea takes a sound wave as input and outputs its Fourier transform along the auditory nerve.

Upon entering the brainstem, the auditory nerve bifurcates and innervates the two divisions of the cochlear nucleus: the dorsal cochlear nucleus (DCN) and the ventral cochlear nucleus (VCN) (Cajal 1911; de Nó 1933). Synapses of auditory nerve fibers within the cochlear nucleus are spatially organized by frequency creating a “tonotopy”

within both divisions (Rose et al. 1959). Higher frequencies are represented more medially and dorsally while lower frequencies are represented more laterally and ventrally in both the VCN and DCN (Rose et al. 1959; Muniak et al. 2013). The work in this thesis takes advantage of this parallel processing and leverages the well-known tonotopy in its experimental designs.

The main projection from the VCN travels through the ventral acoustic stria, also known as the trapezoid body, to the contralateral inferior colliculus (IC) (Osen 1972; Roth et al. 1978) with additional projections traveling through the ventral acoustic stria to the ipsilateral lateral superior olivary nucleus (LSO) and the contralateral and ipsilateral medial superior olivary nucleus (MSO) (Warr 1966; Osen 1969b; Cant & Casseday 1986). The main projection from the DCN also projects to the contralateral IC, but travels through the dorsal acoustic stria (Adams 1979; Beyerl 1978). The IC projects to the medial geniculate body (MGB), the main auditory relay center form the thalamus to the auditory cortex (Powell & Hatton 1969). The MGB also receives additional input directly from the DCN via the dorsal acoustic stria (Malmierca et al. 2002).

This thesis focuses on the cochlear nucleus, the primary sensory nucleus of the auditory pathway, and in particular the dorsal cochlear nucleus. Early neuroanatomists Ramon y Cajal and Lorente de No noticed the layered structure of the DCN and the presence of small neurons resembling those in the cerebellum and therefore classified them as granule cells. Later anatomical, genetic, and electrophysiological studies unveiled further similarities between the DCN and the cerebellum with Lorente de No going so far as to

call the DCN "the cerebellum of the auditory system" (de Nó 1979). The DCN is one of several cerebellum-like sensory structures found in vertebrates, the general features of which are summarized in the next section.

1.2. General features of cerebellum-like structures

Most vertebrate brains have sensory structures that are architecturally similar to the cerebellum itself. These cerebellum-like structures are found across species and sensory modalities, including the medial octavolateral nucleus (MON) found in the lateral line system of fish (Mccormick 1999), the dorsal octavolateral nucleus (DON) found in the electrosensory system of elasmobranches, and the electrosensory lobe (ELL) found in the electrosensory system of mormyrids and gymnotids in addition to the dorsal cochlear nucleus in the auditory system of mammals. A key feature of all these structures is the presence of a molecular layer, which contains parallel fibers – the axons of granule cells – along with dendrites and cell bodies on which the fibers terminate (**Figure 1.1)** (Bell et al. 2008; Bell 2002). Spiny dendrites of efferent cells and a unique class of inhibitory interneurons – "Purkinje-like" cells – form synapses with parallel fibers (Bell et al. 2008). Both the cerebellum and cerebellum-like structures receive two types of input with one being parallel fibers. While in the cerebellum, climbing fibers convey a second input, primary sensory afferents convey the second input in cerebellum-like structures (**Figure 1.1**, bottom black arrows). Indeed, some authors have suggested that presence of climbing fibers is what distinguishes the cerebellum from cerebellum-like structures (Bell et al. 2008).

Studies performed in cerebellum-like structures in the electrosensory system of fish (the DON of elasmobranches and ELLs of gymnotids and mormyrids) have shown that these structures cancel predictable electrosensory consequences of behavior (Bell 1981; Bodznick & MONTGOMERY 1992; Bastian 1995). While natural behaviors such as ventilation in elasmobranches or the electric organ discharge of the active electrosensory system in both gymnotids and mormyrids cause large responses in passive electroreceptors that would interfere with perception of external fields (MONTGOMERY 1984; Bell & C. J. Russell 1978), output cells are able to selectively cancel these self-generated sensory signals (Bodznick et al. 1992; Bell 1981; Bastian 1995) using predictive information conveyed via parallel fibers (the mechanism of which I go into more detail later), allowing for more efficient processing of behaviorally relevant sensory signals.

The next section describes the anatomy of the DCN and its similarities to the cerebellum, paying particular attention to the granule cell, the cartwheel cell, and the fusiform cell.

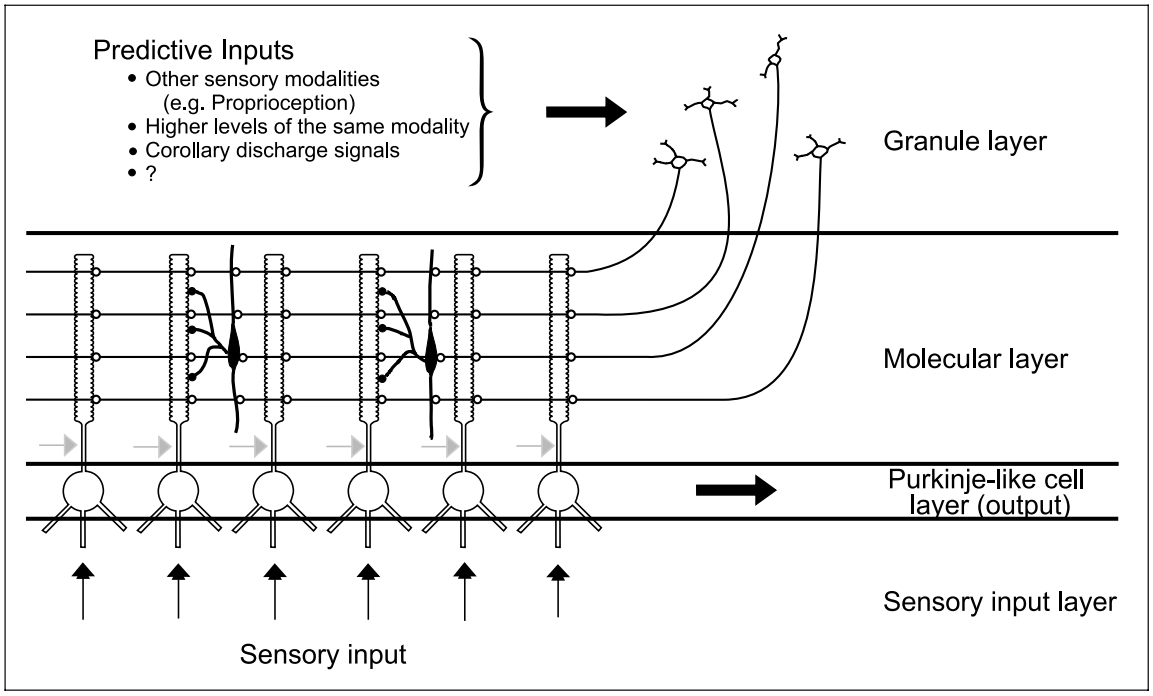

Figure 1.1| General features of a cerebellum-like circuit.

Granule cells convey a variety of input via parallel fibe1rs to a molecular layer where they synapse onto dendrites of principal cells. Principal cells integrate this input with primary sensory input (black arrows). Reproduced from (Bell et al. 2008)

1.3. **Anatomy of the Dorsal Cochlear Nucleus**

The dorsal cochlear nucleus lies on the surface of the brainstem on the floor of lateral recess of the fourth ventricle. It is described as having three main layers: the superficial molecular layer, the fusiform layer, and the deep polymorphic layer (de Nó 1933; Brawer et al. 1974; Kane 1974; Mugnaini & Warr 1980). Cells of the DCN are generally confined to one of these specific layers.

1.3.1. *Granule cells*

As opposed to other cell types of the DCN, granule cells are not confined to a single layer but are instead found in seven distinct granule cell domains that line the borders of both the DCN and VCN (Mugnaini & Warr 1980). In both the mouse and rat, cochlear granule cell bodies are similar in size to their cerebellar counterparts and slightly larger in the cat (10-11 um in the cochlea versus 6 um in the cerebellum) (Mugnaini & Warr 1980). While Cajal thought these cells may be axonless (Cajal 1911), Lorente de No and later anatomists were successful in staining the thin axon of the granule cell (de Nó 1933; Mugnaini & Warr 1980; Mugnaini et al. 1980; Kane 1974; Brawer et al. 1974). These axons run in the DCN molecular layer and are analogous to parallel fibers of the cerebellum (Mugnaini & Warr 1980; Mugnaini et al. 1980; Brawer et al. 1974) (**Figure 1.2**). They additionally run perpendicular to the tontopy of the DCN allowing areas of DCN receiving different auditory nerve fiber input access to the same information conveyed via parallel fibers (Mugnaini & Warr 1980). Similar to their cerebellar

counterparts, dendrites of cochlear granule cells form distinctive claw shapes and participate in complex synaptic structures called glomeruli with mossy fiber endings (Mugnaini et al. 1980; Kane 1974; Brawer et al. 1974; Oertel & Wu 1989; Webster & Trune 1982).

In addition to anatomical similarities to cerebellar granule cells, cochlear granule cells also express GABAA receptor alpha6 subunit, *Math1* and are derived from the rhombic lip (Laurie et al. 1992; Varecka et al. 1994; Fünfschilling & Reichardt 2002; Farago et al. 2006). Their EPSCs are similar in amplitude and decay time as is the presence of NMDA-receptor mediated currents (Balakrishnan & Trussell 2008).

1.3.2. *Fusiform Cells*

Fusiform cells are the efferent cells of the DCN (Cajal 1911; de Nó 1933). Their cell bodies occupy the fusiform layer (Blackstad et al. 1984) and have two main dendrites: an apical dendrite and a basal dendrite (**Figure 1.2**). The apical dendrite extends into the molecular layer where they form synapses with parallel fibers (de Nó 1933; Mugnaini & Warr 1980). Basal dendrites extend into the deeper layer of the DCN where they form synapses with auditory nerve fibers (de Nó 1933). Axons of fusiform cells run through the dorsal acoustic stria and eventually terminate in the inferior colliculus, mainly the central nucleus of the inferior colliculus (Fernandez & Karapas 1967; D. Ryugo & Willard 1985).

Fusiform cells are frequency tuned and follow the tonotopy of the DCN previously described: cells with higher characteristic frequencies (CFs) are located medially to those with lower CFs. Interestingly, a subset of fusiform cells of the decerebrate cat are excited only by CFs played at sound pressure levels near their threshold while higher amplitudes inhibit the cell. This is termed a "Type IV" response. These responses are found much less commonly in rodents where fusiform cells are excited by their CF at threshold and have higher excitatory responses at higher sound amplitudes along with responding to a broader range of frequencies, a "Type III" response (K. A. Davis et al. 1996; Ma & Brenowitz 2012).

In addition to receiving excitatory input from granule cells and auditory nerve fibers, fusiform cells additionally receive inhibitory input from an interneuron that closely resembles Purkinje cells, the cartwheel cells.

1.3.3. *Cartwheel Cells*

Cell bodies of cartwheel cells are located on the border of the fusiform layer and the molecular layer (Berrebi & Mugnaini 1991). They have spiny dendrites that extend into the molecular layer (Brawer et al. 1974; Wouterlood & Mugnaini 1984; Zhang & Oertel 1993) where they receive excitatory input from parallel fibers (**Figure 1.2**) (Hirsch & Oertel 1988; Zhang & Oertel 1993; Manis et al. 1994; K. A. Davis & Young 1997). Cartwheel cell axons form synapses with fusiform and other cartwheel cells (Berrebi & Mugnaini 1991; Zhang & Oertel 1993; Manis et al. 1994; Golding & Oertel 1997). There

is no evidence for direct input via auditory nerve fibers to cartwheel cells though in anesthetized and decerebrate preparations, cartwheel cells do show an excitatory response to both tones and noise, albeit weakly and with longer first spike latencies than fusiform cells (Parham & D. O. Kim 1995; K. Davis & Miller 1996; Ma & Brenowitz 2012).

Cartwheel cells share many genetic similarities to Purkinje cells. Cerebellin and the calcium binding protein PEP-19, both Purkinje cell markers, are also expressed in cartwheel cells(Mugnaini & Morgan 1987; Mugnaini et al. 1987). Berrebi et al. went a step further by studying cartwheel cell phenotypes in mouse mutants that show severe degeneration of Purkinje cells: *Lurcher, Purkinje cell degeneration (PCD),* and *staggerer* (Berrebi & Morgan 1990). Degeneration of Purkinje cells in these mouse lines are caused by independent mutations in different genes located on separate chromosomes (Zuo et al. 1997; Fernandez-Gonzalez & La Spada 2002; Hamilton et al. 1996). All three mouse lines also show severe degeneration of cartwheel cells in the DCN while sparing other cell types in the brainstem further demonstrating genetic similarities between the two cell populations (Berrebi & Morgan 1990).

Electrophysiologically, cartwheel cells are easily identified by the presence of two distinct spike types: the simple spike and the complex spike. No other cell type in the cochlear nucleus displays two spike types, although Purkinje cells also fire a simple and complex spike. Complex spikes of cartwheel cells are defined as high frequency sodium dependent action potentials superimposed on a slower calcium dependent depolarization (Zhang & Oertel 1993; Manis et al. 1994; Golding & Oertel 1997) and are easily

identified both intracellularly and extracellularly (Portfors & P. D. Roberts 2007; Ma & Brenowitz 2012). While Purkinje cells fire complex spikes only in response to climbing fiber input, cartwheel cells lack any such input and instead fire complex spikes spontaneously or in response to parallel fibers (Golding & Oertel 1997; Tzounopoulos et al. 2004; Zhang & Oertel 1993). What role complex spikes play in the function of DCN is poorly understood, indeed the role of cartwheel cells in shaping the output of DCN is still not known.

1.3.4. *Additional cell-types in the dorsal cochlear nucleus*

Similarities to the cerebellar circuit are not restricted to granule cells and cartwheel cells. Golgi cells, unipolar brush cells, and stellate cells that are analogous to their cerebellar counterparts are also present (**Figure 1.2**). Much like in the cerebellum, cochlear Golgi cells are located in the granule cell domains and form inhibitory synapses with granule cells (Mugnaini et al. 1980). Unipolar brush cells (UBCs) are also found in the granule cell domains but form excitatory synapses with granule cells (Floris et al. 1994; Borges-Merjane & Trussell 2015). Both cerebellar and cochlear UBCs receive mossy fiber input and express calretinin and metabotropic glutamate receptors (Floris et al. 1994). Similar to cerebellar stellate cells, cochlear stellate cells are located in the molecular layer where they receive excitatory input on their relatively smooth dendrites via parallel fibers and are electrically coupled to each other via gap junctions (Wouterlood et al. 1984; Apostolides & Trussell 2014). They are GABAergic and form

synapses with both cartwheel and fusiform cells (Wouterlood & Mugnaini 1984; Mugnaini 1985).

Fusiform cells are not the only DCN cell type that receives auditory nerve input. Vertical cells (also called tuberculoventral cells) are located in the polymorphic layer where they receive input from auditory nerve fibers (De No 1981) and send inhibitory input to fusiform cells (Voigt & Young 1980; Voigt & Young 1990). They respond to a narrow range of frequencies and, much like fusiform cells, are arranged tonotopically and inhibit fusiform cells with similar CFs
(Young & Voigt 1981; Wickesberg & Oertel 1988).

Taken together, the anatomic, genetic, and electrophysiologic studies provide compelling evidence that the dorsal cochlear nucleus is indeed the "cerebellum of the auditory system." However, an important component of the description of DCN circuitry was lacking in the early characterizations of the DCN: the source of mossy fiber input to the granule cell domains. Mugnaini et al. wrote, "Little that is known about the sources of the granule cell afferents is definite, except that they most probably do not originate from the spiral ganglion," (Mugnaini & Warr 1980). Where, then, do the mossy fibers come from?

1.4. **Mossy fiber input to granule cell domains**

Anatomical and electrophysiological studies have uncovered numerous sources of mossy fiber input to the granule cell domains of the DCN. Many of these inputs come from a variety of non-auditory sources: the dorsal column nuclei, the spinal trigeminal nucleus, the pontine nuclei, and other areas that are known centers of somatosensory and motor information. This suggests that the integration of two streams of information – auditory via auditory nerve fibers and non-auditory via mossy fibers – is important to the function of the DCN. In this section, I describe the evidence for sources of mossy fiber input.

The dorsal column nuclei, consisting of both the cuneate and gracilis, transmit information related to fine touch and proprioception from the body, back of the head, pinna, and limbs to the thalamus. Itoh et al. showed via an aneterograde and retrograde wheat germ agglutinated horseradish peroxidase study in the cat that the dorsal column nuclei also send information to the DCN (Itoh et al. 1987). In the same year, Weinberg and Rustioni had congruous results in a similar study in the rat (Weinberg & Rustioni 1987). Both studies found that the large majority of inputs to the DCN came from the ipsilateral dorsal column nuclei with a much smaller number coming from the contralateral side. Further, units in the DCN that had responses to auditory stimuli also showed robust responses to electrical stimulation of the dorsal column nuclei (Saade et al. 1989). In 2011, Kanold et al. (Kanold 2001) showed responses to tones increased or decreased when preceded by stimulation of the dorsal column nuclei. However, how this integration between auditory and non-auditory stimuli manifests itself in natural behaviors has never been shown.

The dorsal root ganglion of the C2 represents one of the best characterized inputs to the DCN. Anterograde and retrograde labeling shows that the granule cell domain receives a direct projection from the dorsal root ganglion (DRG) of the C2 (Zhan & Pongstaporn 2006). Direct electrical stimulation of the DRG of C2 caused and evoked field potential in DCN and an inhibition of DCN output cells (Kanold 2001). Kanold et al. (Kanold 2001) went further and stimulated individual nerve branches of C2 in a decerebrated cat to see if they could determine which specific branch contributes the most to the evoked potential. Branches of C2 go on to innervate the muscles and skin of the caudal pinna, back of the head, and the neck. The largest evoked potentials in the DCN came from stimulation of branches that innervate the skin and muscles of the pinna while smaller evoked potentials were observed in branches that innervated the back of the head. No evoked potentials were observed in branches of C2 that innervated the neck. Armed with this information, they recorded from DCN fusiform and cartwheel cells while applying gentle stretch to the pinna. Fusiform cells showed an inhibition to stretching similar to that seen in electrical stimulation of the DRG while cartwheel cells showed an excitation. Since cartwheel cells provide known inhibitory input to fusiform cells, this suggests that cartwheel cells may be responsible at least in part to the inhibition observed. It should be noted that these responses need not be mediated directly from mossy fiber inputs from the DRG but can also be mediated the dorsal column nuclei which receives inputs from the DRG of C2.

The spinal trigeminal nucleus (sp5) represents another source of somatosensory input to the DCN. Somatosensory information from the face, jaw and intraoral structures, as well as whiskers in rodents, is conveyed via sp5. In their horseradish peroxidase study, Itoh et al. also observed numerous mossy fiber inputs to the granule cell domain from the ipsilateral sp5 in addition to the dorsal column nuclei (Itoh et al. 1987). Retrograde studies showed inputs come from throughout the rostral-caudal extent of this large nucleus (Haenggeli & Pongstaporn 2005; Zhou & Shore 2004). Stimulation of the sp5 caused evoked potentials in DCN in a decerebrate preparation of the cat (Young et al. 1995). Similar to the stimulation of the dorsal column nuclei, DCN units showed both increases and decreases to their tone response when the tone was preceded by stimulation of sp5 (Shore 2005a). Following a similar pattern of the dorsal column nuclei where the dorsal root ganglion itself also sends mossy fiber input, the trigeminal ganglion itself is a source of mossy fiber input (Shore et al. 2000), and DCN units respond to electrical stimulation of the trigeminal ganglion alone (Shore 2005a). Experiments analogous to those Kanold et al. performed in 2001 (Kanold 2001) were never performed in structures innervated by the trigeminal nerve such as stretching of the jaw or movement of the whiskers. It is therefore still unknown what structures of the face are the main contributors to mossy fiber input to the DCN.

There is anatomical evidence for mossy fiber input from the pontine nuclei (Ohlrogge et al. 2001), lateral reticular formation (Zhan & D. K. Ryugo 2007), vestibular nerve (Burian et al. 1989), and auditory cortex (Weedman 1996) to the granule cell domain of the DCN however no further electrophysiological experiments have been

performed. It is therefore yet to be determined how activation of these areas affects responses in DCN units. Finally, in contrast to what was reported by Mugnaini et al., auditory nerve fibers do directly innervate the granule cell domain (Brown et al. 1988) which may explain why cartwheel cells have auditory responses while only receiving parallel fiber input.

While there is ample evidence for the identity of mossy fiber input to the granule cell domain of the DCN, many of the experiments described were done in cats, rats, or guinea pigs, and none were performed in mice, a commonly used laboratory animal in part due to its genetic tractability. Further, the electrophysiological experiments were performed in decerebrate or anesthetized animals where sources of mossy fiber input were electrically stimulated or unnaturally stretched. The work done in this thesis attempts provide additional insight into the roles played by mossy fibers by engaging mossy fiber input to DCN in awake mice performing natural behaviors.

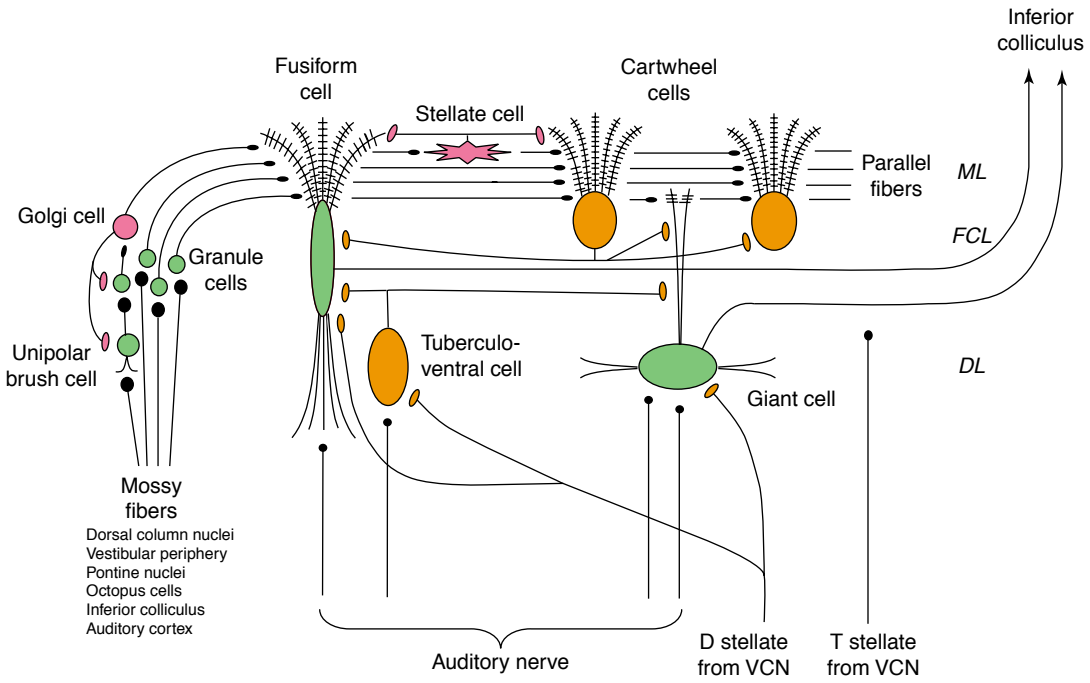

Figure 1.2| Circuitry of the DCN resembles a cerebellum. Granule cells receive a rich variety of input conveyed via mossy fibers. Axons of granule cells (parallel fibers) synapse onto spiny dendrites of fusiform cells and cartwheel cells in the molecular layer. Golgi cells, UBCs and stellate cells also have similar connectivity and properties that resemble their cerebellar counterparts. Reproduced from Oertel and Young, 2004.

1.5. **Plasticity at parallel fiber synapses in the dorsal cochlear nucleus**

Parallel fiber synapses to cartwheel cells and fusiform cells are not static but can strengthen or weaken. In mouse brain slices, Fujino et al. (Fujino & Oertel 2002) showed that when parallel fibers are stimulated at high frequency (100 Hz), amplitude of EPSPs caused by parallel fiber stimulation in both cartwheel cells and fusiform cells increased. Conversely, low frequency stimulation (1 Hz) caused a decrease in EPSP amplitude. When auditory nerve fibers were stimulated instead, no change in amplitude of EPSPs evoked by auditory nerve stimulation occurred. Tzounopoulos et al. (Tzounopoulos et al. 2004) showed in mouse brain slices that repetitions of an EPSP in cartwheel cells caused by stimulation of parallel fibers followed 5 ms later by a spike caused a decrease in amplitude of the EPSPs, while no change occurred in EPSP amplitude if spikes preceded EPSPs (an anti-Hebbian learning rule). In fusiform cells, the same experimental protocol caused increases in EPSP amplitude when spikes followed EPSPs and decreases in EPSP amplitude when spikes preceded EPSPs (a Hebbian learning rule) (Tzounopoulos et al. 2004). Interestingly, when cholinergic inputs to the DCN were stimulated prior to performing the EPSP-spike pairing protocol, the fusiform cell learning rule flipped from Hebbian to anti-Hebbian (Zhao & Tzounopoulos 2011). Similar to results obtained by Fujino et al., no change in EPSP amplitude occurred when EPSPs were produced by stimulation of auditory nerve fibers (Zhao & Tzounopoulos 2011) showing that the plasticity to fusiform cells is specific to parallel fiber synapses. Depressions of parallel fiber synapses onto both cartwheel and fusiform cells are mediated by endocannabinoid signaling (Tzounopoulos et al. 2007; Zhao & Tzounopoulos 2011).

In vivo experiments performed in anesthetized guinea pigs played sounds in conjunction with stimulation of sp5 (Koehler & Shore 2013). When sp5 was stimulated prior to playing the sound, DCN units that displayed type III and type IV responses (putative output cells) showed a decrease in response to sound when played without stimulation. The mechanism of this depression is not known. Further, while plasticity in DCN can be induced via electric stimulation in both slice and anesthetized preparations, it is not known if natural behaviors paired with sound induce similar changes.

1.6. **What's a cerebellar circuit doing in the auditory system?**

The complexity of the DCN circuitry was readily apparent to even the early neuroanatomists. Lorente de No theorized in 1979 that the function of the DCN was "to decide which paths should be open and which ones should be closed to cochlear impulse (*i)* depending upon the constellation of impulses that accompany (*i*) and upon the constellation of impulses that preceded (*i*)" (de Nó 1979). Almost forty years later, the nature of these impulses to DCN is better characterized but what role this complex cerebellum-like structure plays in auditory processing remains a mystery (Oertel & Young 2004). Two main hypotheses for the role of multisensory integration and the cerebellum-like circuit have developed: localizing sounds in the vertical plane and cancelling self-generated noise.

Hypothesis 1: The dorsal cochlear nucleus relays information related to sound localization in the vertical plane

Sounds originating on either side of the head reach the two ears at different times and at different levels. Therefore interaural time differences (ITDs) and interaural level differences (ILDs) can be used to localize sounds in the horizontal plane. This is not the case however for sounds located along the midline. In this case, sounds located at two different points along the vertical plane along the midline reach the ears at the same time and at the same levels. How then are animals able to distinguish the location of these two sounds? One hypothesized method is that the auditory system takes advantage of differing monaural spectral cues caused by the filtering of sounds through the pinna prior to reaching the tympanic membrane (Gardner 1973). Sounds located at different vertical positions are filtered differently due to the asymmetrical shape of the pinna (Musicant 1990; Rice et al. 1992). The ratio of the spectra of the filtered sound to the original sound is called the head-related transfer function (HRTFs). HRTFs in the cat and mouse show a distinctive notch in their HRTFs that change in frequency location depending on the vertical location providing a potential source of information for localization in the vertical plane (**Figure 1.3**) (Rice et al. 1992; Lauer et al. 2011).

Reiss et al. showed that neurons with type IV responses in the cat DCN are particularly sensitive to notches whose rising spectral edge fell on the neurons' characteristic frequency (Reiss 2005). Behavioral experiments done in cats, in which the dorsal acoustic stria was cut, effectively lesioning the DCN while sparing the VCN, showed a

deficit in the ability to orient to sounds located in the vertical plane but not in the horizontal plane (May 2000). For those animals with mobile pinnae, it is also important to know the pinnae's position since HRTFs are pinnae position dependent (Young et al. 1996). Electrophysiological experiments have already been shown that the DCN has access to information related to pinna position (Kanold 2001). However, Oertel et al. (Oertel & Young 2004) recognized that since fusiform cells are sensitive to specific frequencies and not to specific locations in space, it is unclear how a correction related to pinna movement would occur in the DCN. Further, studies done in mice, whose DCN output neurons display type III rather than type IV responses, have shown that they localize sound poorly in the vertical plane, but not in the horizontal plane, suggesting a different functional role for the DCN (Lauer et al. 2011). Studies based on an analogous cerebellum-like structure have led to an alternative hypothesis on the functional role of DCN.

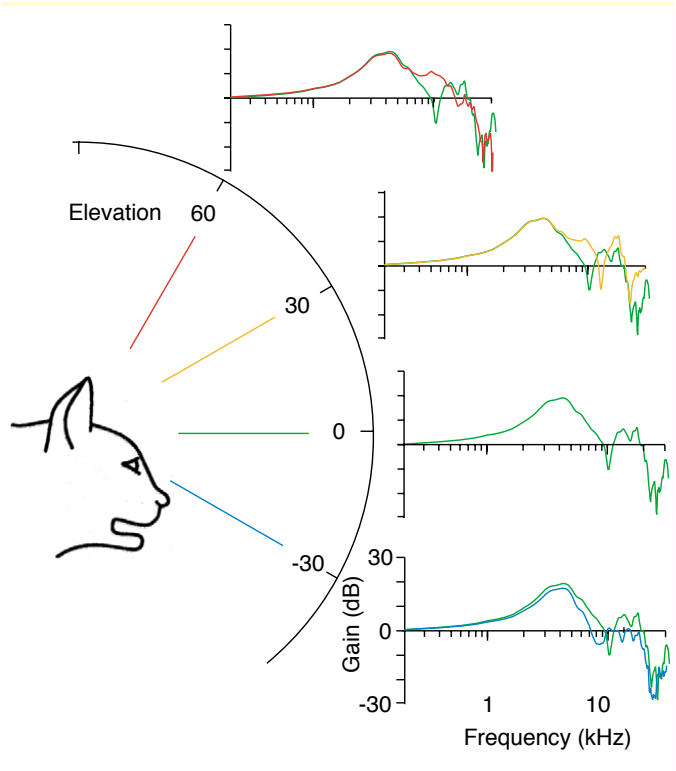

Figure 1.3| Notch frequencies of HRTFs shift as a function of sound source elevation. HRTFs taken at different elevations are shown in the cat with HRTF at 0 degrees reproduced on each plot for reference. Location of notch frequency systematically changes with sound source elevation with higher notch frequencies at higher elevations and lower notch frequencies at lower elevations. Reproduced from Oertel and Young, 2004.

Hypothesis 2: The DCN cancels self-generated sounds

How to distinguish behaviorally relevant sensory input from those caused by the animal's own behavior is a general problem sensory systems must solve. Since primary sensory afferents respond regardless of source, animals must be able to disambiguate the incoming information. From their experiments in the visual system, Von Holst and Mittelstaedt (Holst & Mittelstaedt 1950) and Roger Sperry (Sperry 1950) proposed that information about the animal's own movement ("efference copy" or "corollary discharge") is used to predict the sensory consequences of those movements (termed "reafference"). Subsequent studies have characterized corollary discharge and sensory prediction pathways in many systems including the vestibular (Roy & Cullen 2001; Roy & Cullen 2004) and lateral line (B. L. Roberts & I. J. Russell 1972) systems.

One of the best-studied systems is the electrosensory system of weakly electric fish, particularly in mormyrids. These fish have a passive electrosensory system that detects low frequency external electric fields such as those caused by contraction of muscles from other animals. They have also developed an "active" electrosensory system where an electric organ, a modified muscle, emits a weak, high frequency electric pulse known as an electric organ discharge (EOD) that is used to detect nearby objects. Electroreceptors of the passive system not only respond to low frequency external electric fields, but also have robust responses to the high frequency pulse emitted from the electric organ (Bell & C. J. Russell 1978). The amplitude of these responses can change

over time with changes in water conductivity. Further, the animal's own movements cause changes in electroreceptor position relative to the electric organ which also causes large changes in electroreceptor firing (Engelmann et al. 2008; Sawtell & Williams 2008). How then is the animal able to tell the difference between electric fields occurring externally in the water and those produced by its own EOD?

The first stage of electrosensory processing occurs in the electrosensory lobe (ELL), which shares remarkable similarities to the DCN. The ELL is also a cerebellum-like structure containing many analogous cells to the cerebellum, including granule cells, Golgi cells, UBCs, stellate cells and Purkinje-like cells. Output cells of ELL integrate primary sensory input from their basal dendrites with parallel fiber input on their apical dendrites. Granule cells in the ELL receive mossy fiber input from both somatosensory and motor areas (Srivastava 1977; Szabo et al. 1990; Bell et al. 1992; Sawtell 2010; Requarth & Sawtell 2014). Experiments performed in paralyzed fish, in which the electric organ did not discharge but motor commands related to the EOD were preserved, paired an electric field stimulus with EOD motor commands (Bell 1981). In output cells, there was little to no response to the command alone (**Figure 1.4,** top). When the stimulus was paired to the command, the response to the stimulus decreased over time and the response to the command alone resembled a negative image of the initial response to the stimulus (**Figure 1.4**, bottom). While paired, the addition of the negative image minimizes the effect of the predictable input. Similar results were observed when stimulus was paired to position of the tail (Requarth et al. 2014) or motor commands related to movement of the tail (Requarth & Sawtell 2014). *In vivo*, *in vitro*, and

modeling experiments have demonstrated that the formation of negative images is due at least in part to an anti-Hebbian learning rule, similar to that found in DCN, at parallel fiber synapses in ELL (Bell et al. 1993; Bell, Han, et al. 1997; P. D. Roberts & Bell 2000). Taken together, these studies show that the ELL acts as an adaptive filter that can predict and cancel self-generated electrosensory stimuli.

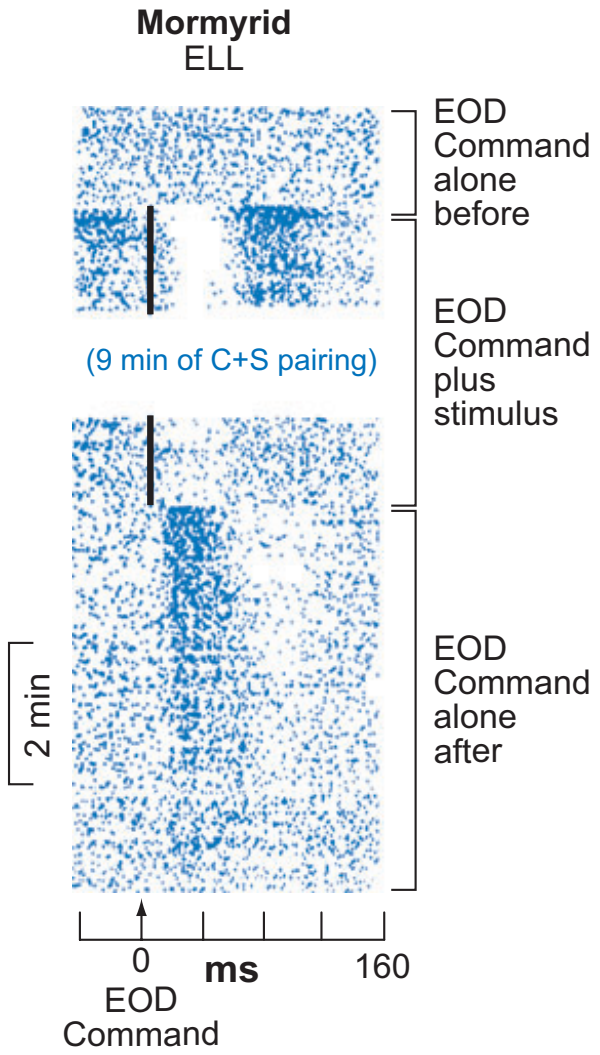

Figure 1.4| Sensory cancellation and negative image formation in mormyrid ELL. Output cells of ELL showed little to no response to the EOD command alone (occurring at time 0, arrow). An electrosensory stimulus was then paired with the EOD command. Responses to the electrosensory stimulus decreased over 9 minutes of pairing. Responses to the EOD command alone after pairing was opposite the initial response to the stimulus, or a "negative image." Reproduced from Bell et. al 2008.

Can the DCN play a similar role as an adaptive filter in the auditory system? Somatosensory and motor information conveyed via its mossy fibers to cochlear granule cells can potentially be used as predictive inputs to cancel self-generated sounds. Further, anti-Hebbian plasticity found at parallel fiber synapses would allow changes to sensory predictions similar to those found in ELL. While previous experiments have shown that repeated pairing of stimulation of somatosensory inputs with sounds lead to both increases and decreases in response to the sound alone in an anesthetized animals (Koehler & Shore 2013) these non-specific changes would not be helpful in only cancelling self-generated sounds while remaining sensitive to external sounds. These changes could be due to the unnatural electrical stimulation or anesthetized state of the animal and may not be representative of what occurs in a normal state. The work done in this thesis will use an awake mouse preparation performing natural behaviors to test the hypothesis that the DCN predicts and cancels self-generated sounds. It further lays the groundwork for future experiments that take advantage of the genetic and molecular techniques available in the mouse to better study the importance of multisensory integration and the cerebellum-like circuit.

2. CHAPTER 2: THE DORSAL COCHLEAR NUCLEUS SUPRRESSES SELF-GENERATED SOUNDS

2.1. Introduction

The ability for sensory systems to distinguish external stimuli from those caused by the animal's own movements is an important function of the nervous system. Primary sensory afferents typically respond to stimuli regardless of their source, hence central mechanisms are required to make the distinction between externally- versus self-generated inputs. Von Holst and Mittelstaedt and Roger Sperry independently theorized that signals related to the animal's own movements, e.g. proprioception or motor corollary discharge, are used to predict and cancel self-generated input (Holst & Mittelstaedt 1950; Sperry 1950). In their experiments, Von Holst and Mittelstaedt placed blowflies into cylinders with painted vertical lines. When the cylinder was rotated to the left, flies flew left, and vice versa when the cylinder was rotated to the right. This had the consequence of stabilizing the flies' visual fields and was called the optomotor response. However when the heads of blowflies were rotated 180 degrees, effectively switching the locations of the left and right eye, flies flew in the opposite direction of the cylinder rotation. Further, flies continuously flew in circles within the stationary cylinder and only had normal "voluntary" flight in a visually homogeneous container. They hypothesized that flies were comparing visual input with the predicted sensory

consequences of flight (coming from an "efference copy"). Under normal circumstances this would cause negative feedback to stabilize the visual field, but after inverting the head caused positive feedback making the fly continuously circle.

Evidence of using predictive input from the animal's own movements to cancel sensory consequences of those movements has since been observed in many sensory systems. Roy et al. in the primate vestibular system (Roy & Cullen 2001) found that cells in the vestibular nuclei respond selectively to passive movements applied to the head and have little to no response to active head movements. Later experiments dissociated the actual head movement that occurs from the intended movement conveyed by motor signals (Roy & Cullen 2004). The authors observed that when neck proprioceptive information was vastly different from what would be expected from the motor signals, cells in the vestibular nuclei had a strong response and it was only when neck proprioceptive information matched what would be expected from motor input did sensory cancellation occur. Cerebellum-like structures in the electrosensory system of weakly electric fish are able to use proprioceptive and motor information relayed by mossy fibers to its granule cells to predict and cancel sensory consequences related to their own behaviors, the mechanism of which I go into greater detail in later chapters.

The dorsal cochlear nucleus (DCN) is one of the first stages of auditory processing. Its cerebellum-like circuitry integrates multiple sources of somatosensory and motor input conveyed via mossy fibers with auditory information conveyed via auditory nerve fibers. Why is such a cerebellum-like circuit found in the auditory

system? While it has been hypothesized that the function of the DCN is to predict and cancel self-generated sounds, the cancellation of self-generated sounds has never been shown experimentally.

In this chapter, I develop a novel awake preparation in mice in which to test this hypothesis. I will first show that a natural repetitive behavior, licking, causes a consistent self-generated noise. To determine if these self-generated noises are relevant to the mouse auditory system, I take advantage of the auditory nerve fiber bifurcating and sending the same information to both the DCN and the ventral cochlear nucleus (VCN). Since the VCN does not share the cerebellum-like circuitry of the DCN, I expected cells in the VCN to respond to the self-generated licking sounds if indeed the licking sounds were within the hearing range of mice. I then show that DCN cells have weak responses to self-generated lick sounds and that this is not due to differing auditory receptive fields or to an overall suppression in auditory responses while licking.

2.2. **Results**

2.2.1. *Licking behavior is a source of self-generated sounds*

Guided by previous studies of cerebellum-like sensory structures in fish (Bell et al. 2004; Bell et al. 2008), we set out to test the hypothesis that DCN functions to cancel out self-generated sounds. To this end we developed a preparation to study neural responses to self-generated sounds in awake, behaving animals. We chose licking behavior in mice

because it is stereotyped and repetitive, can be elicited in head-fixed animals during electrophysiological recordings, and is likely to engage somatosensory mossy fiber-granule cell inputs to DCN (Haenggeli & Pongstaporn 2005; Shore et al. 2000; Zhou & Shore 2004).

We found that rhythmic licking generates sounds with stereotyped spectral and temporal profiles (**Figure 2.1**). Licking sounds were characterized by an initial increase in loudness which had maximum power at 2 kHz just before tongue contact with the water spout (**Fig 2.1a i, Figure 2.1b**) and a second, larger increase in loudness that peaks 50-100 ms after tongue contact. This second increase had two frequencies with local power maximums: 8 kHz and 30 kHz. Since the loudest components of the lick sound peaked well after contact with the lick spout (**Figure 2.1a**), and in fact peaked after the tongue was no longer in contact with the lick spout in a few animals (**Figure 2.1a iv**), self-generated sounds due to licking do not appear to be primarily due to tongue contact with the water spout.

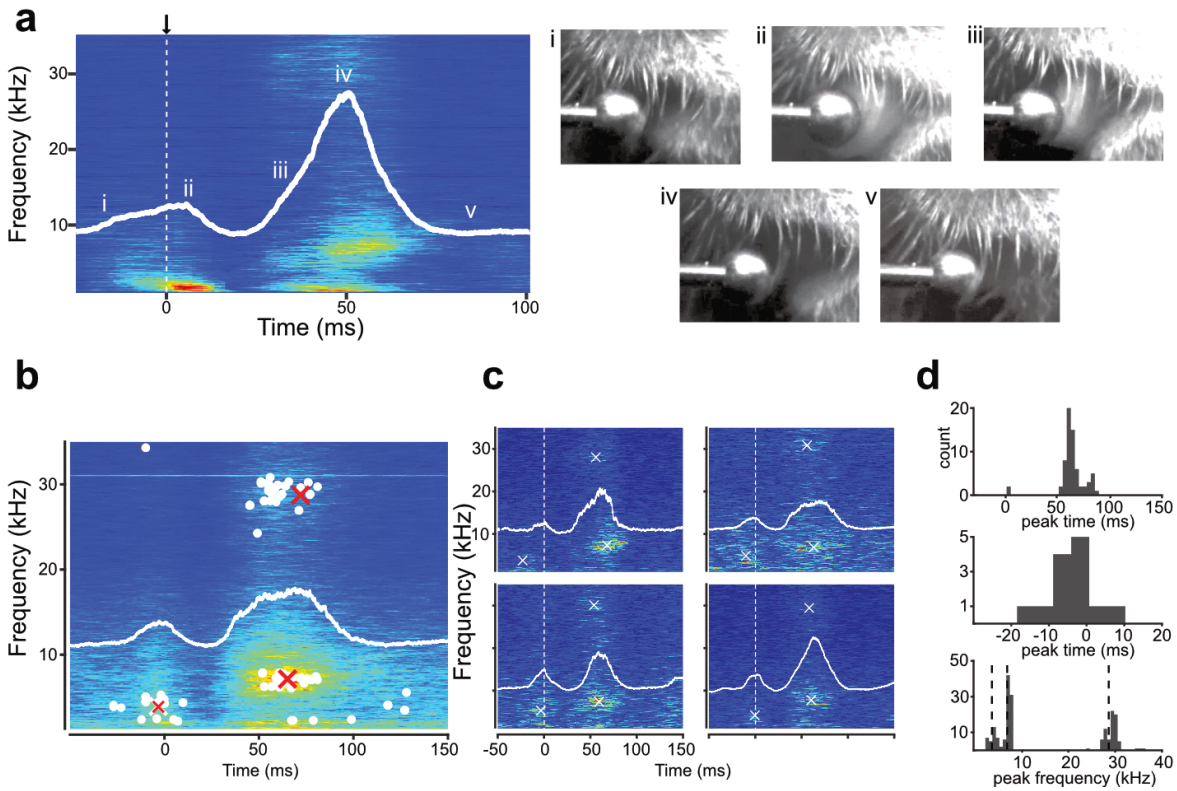

Figure 2.1| Licking is a source of self-generated sounds. **a,** Average spectrogram of self-generated sounds during licking for a representative mouse (n=900 licks). Arrow and dotted line indicate time of tongue contact with the lick spout. Solid white line indicates the root mean squared (RMS) amplitude of the sound. Corresponding video frames over the duration of the lick cycle: i. jaw opening, ii. tongue protrusion and lick spout contact, iii-iv. tongue retraction, v. jaw closure. **b,** The average spectrogram of licking sounds across mice (n = 20) triggered on tongue contact with the lick spout. W*hite circles* show the time-frequency peaks of the spectrograms of each individual mouse. *Red crosses* show time-frequency peaks of the average spectrogram. **c,** Four examples of lick-triggered spectrograms from individual mice. *White crosses* show time-frequency peaks. **d,** *Top,* Histogram of the timing of the main peak of the licking sound with respect to onset of tongue contact with the lick spout. *Middle* Histogram of the timing of the main peak of the licking sound with respect to offset of tongue contact with the lick spout. *Bottom,* Histogram of the frequencies at which peaks in the lick-triggered spectrogram occur, showing that the lick-triggered sound consists of three distinct spectral peaks.

2.2.2. *Self-generated lick sounds are suppressed in the DCN*

We next sought to determine whether licking sounds are an actual source of interference for the mouse auditory system and, if so, whether such sounds are cancelled in DCN. To this end, we compared responses during licking in well-isolated single-units in the VCN and DCN. Recording locations were judged based on characteristic reversals of tonotopy (Luo et al. 2009; Muniak et al. 2013) at the DCN/VCN border and verified histologically by iontophoresis of biocytin (**Figure 2.2**). Upon entrance into DCN, multiunit activity was strongly driven by tones between 5 and 15 kHz (**Figure 2.2a**). As electrodes were advanced ventrally, multiunit activity was driven by lower frequency tones (**Figure 2.2a,** 50-400 μm). Approximately 450-600 μm below the surface of the DCN, multi-unit activity was suddenly driven by higher frequency tones and signaled entrance into VCN. As electrodes were advanced through VCN, multiunit activity was driven by progressively lower tones, similar to that seen in DCN (**Figure 2.2a,** 600-900μm). These observations are consistent with previous work mapping tonotopy of the two divisions of the cochlear nuclei (Luo et al. 2009; Muniak et al. 2013).

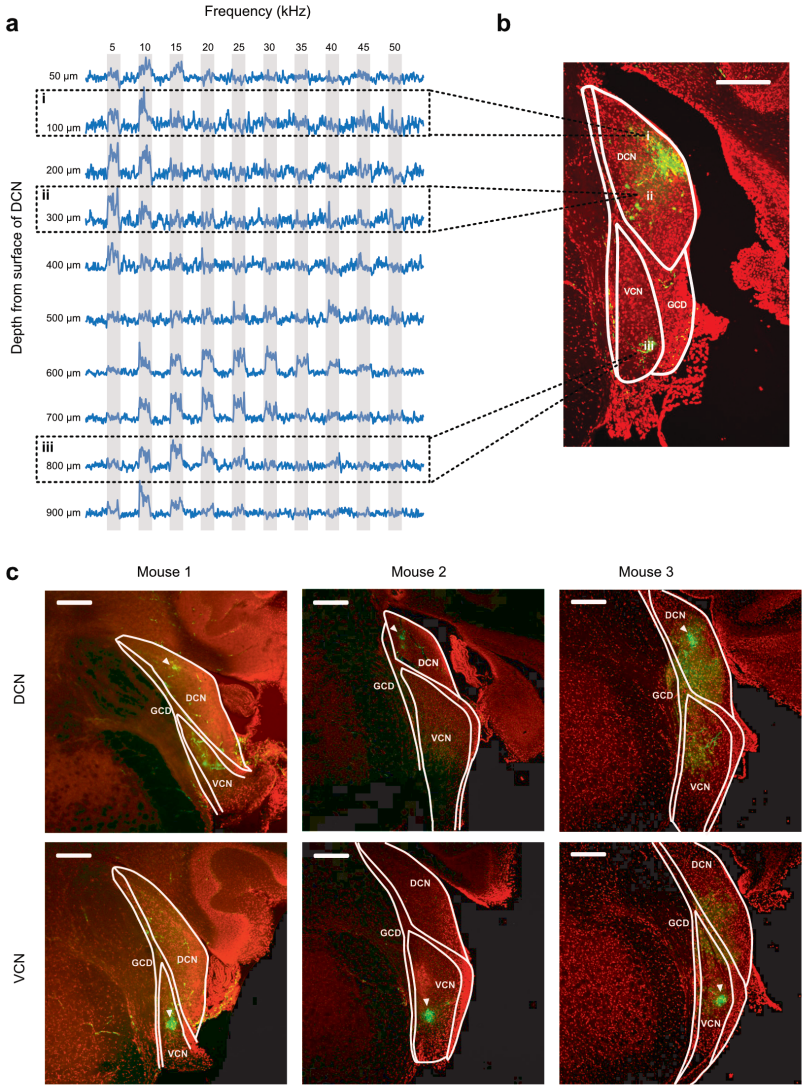
a
Frequency (kHz)
5
10
15
20
25
30
35
40
45
50
Depth from surface of DCN
50 µm
i
100 µm
200 µm
ii
300 µm
400 µm
500 µm
600 µm
700 µm
iii
800 µm
900 µm
b
i
DCN
ii
VCN
GCD
iii
c
Mouse 1
Mouse 2
Mouse 3
DCN
VCN
GCD
DCN
VCN

Figure 2.2| Identification and verification of recording sites in VCN and DCN. **a**, Rectified extracellular multiunit activity (each record is the average of 15 presentations) recorded on an electrode penetration through the auditory brainstem in response to 100 ms tones ranging in frequency from 5-50 kHz (gray bars). As the electrode passes through DCN the frequency evoking the largest multiunit response smoothly decreases. DCN units were isolated in DCN at depths between 100 um (i) and 300um (ii). A sudden increase in frequency (occurring between depths of 400 um and 600 um) indicated entrance into VCN. VCN units were isolated at depths between 800 um (iii) and 1000 um. **b**, Histological verification of recording sites in the same animal as the multiunit recordings shown in **a**. Biocytin (green) was iontophoretically injected at depths of 100 um (i) and 800 um (iii). Scale bar = 200 um. **c**, Iontophoretic injections of biocytin at recording sites (arrows) in DCN (top) and VCN (bottom) in 3 additional animals. Scale bars = 200 um. DCN – Dorsal Cochlear Nucleus, VCN – Ventral Cochlear Nucleus, GCD – Granule Cell Domain of Dorsal Cochlear Nucleus

Though unambiguous criteria for linking unit response properties with morphological cell classes have not yet been established for awake DCN (Ma & Brenowitz 2012), several properties of recorded units indicate that they correspond mainly to output cells. Only units that lacked complex spikes were included in this analysis since only cartwheel cells display complex spikes in DCN (Manis et al. 1994; Zhang & Oertel 1993; Portfors & P. D. Roberts 2007; Ma & Brenowitz 2012). Response type II, type I/III, and type III-i cells are strongly associated with vertical (also known as tuberculoventral) cells (Rhode 1999; Young & Brownell 1976; K. A. Davis et al. 1996). These cells are inhibitory interneurons in deep layers of DCN that also receive input from auditory nerve fibers. One criterion for response type II and type III cells is spontaneous rates below 2.5 Hz (Young & Brownell 1976), however all simple-spiking only units recorded had spontaneous rates above 2.5 Hz, with a mean of 46.4 Hz ± 26.2 Hz, therefore excluding these cells as response type II and type I/III cells (**Figure 2.3a**). One criterion for type III-i cells are inhibitory responses to noise (K. A. Davis et al. 1996), however all simple-spiking only units recorded had excitatory responses to noise with a mean of 47.2 Hz ± 26.4 Hz (**Figure 2.3b**). Further, higher spontaneous rates and excitatory responses are consistent with responses of

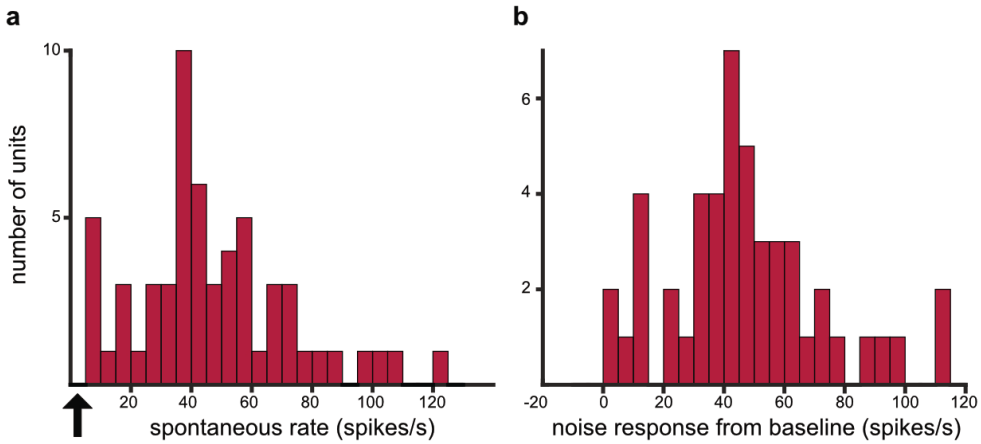

Figure 2.3| Baseline firing and noise-evoked responses in DCN units. a, Histogram of spontaneous firing rates of all units recorded in DCN (n = 65), excluding complex-spiking units. The average spontaneous rate was 46.4 ± 26.2 Hz (mean and s.d.). No DCN units met the criterion for type II and type I/III responses, i.e. a spontaneous rate less than 2.5 Hz (arrow) Type II responses are associated with a major class of DCN interneuron known as vertical cells. **b,** Histogram of responses to noise stimuli in DCN units (n = 48) , excluding units with complex spikes. Stimuli included the mimic of the licking sound (12 dB SPL) and 5-15 kH bandpassed noise (15 dB SPL). Average maximum noise response from baseline was 47.2 ± 26.4 Hz (mean and s.d.). No units showed inhibitory noise responses, a criterion for type III-i response types.

morphologically identified output cells recorded in previous studies (Hancock & Voigt 2002; Young 1980; K. A. Davis et al. 1996).

Since VCN receives direct auditory nerve input but lacks cerebellum-like circuitry, we hypothesized that VCN units should respond to sounds regardless of whether they are self- or externally-generated. Indeed VCN units exhibited an overall elevation of their firing rate during licking (n = 21 units, 16.6 Hz ± 14.6 Hz) and firing rate modulations (n = 21 units, peak-to-trough firing rate: 43.8 ± 26.9 Hz) that were correlated with the amplitude of the licking sound (**Figure 2.3a,b**). Conversely, DCN units exhibited significantly weaker firing rate modulations (n=25 units, peak-to-trough firing rate: 22.5 ± 24.4 Hz for DCN, P=0.0005, Wilcoxon Rank Sum Test, **Figure 2.3c,d**). To further analyze lick responses of VCN and DCN cells, we compared the maximum lick triggered responses and compared them to the average maximum response of 500 shuffled spike trains. The number of standard deviations away from the average response of the shuffled spike trains was called the z-score. Z-scored lick responses for DCN cells were significantly lower than those for VCN cells (n=25/21 units for DCN/VCN, P=0.00002, Wilcoxon Rank Sum Test, **Figure 2.3e**).

Differences in responses in VCN and DCN units during licking could simply be due to differences in auditory response properties of the units we sampled. We evaluated this possibility in a subset of VCN and DCN units by comparing responses during licking to those during delivery of an external sound with temporal and spectral properties that mimicked recorded licking sounds including two separate increases in loudness and

spectral peaks at 2, 8, and 30 kHz similar to those found in recorded lick sounds (**Figure 2.4a**).

The mimic was delivered at 12 dB SPL during periods when the mouse was not licking. Strong responses to the mimic were seen in both VCN and DCN units (**Figure 2.4 c-f**). These responses were not significantly different (peak-to-trough firing rate: n=6; 55.4 ± 72.8 Hz for VCN, and n=13; 63.9 Hz ± 30.2 Hz for DCN, mean ± standard deviation, P=0.32, Wilcoxon Rank Sum Test). Further, VCN units' response to the mimic were highly correlated to their response to licking (n = 6, slope=0.75 $P < 0.001$, $r = 0.95$, linear regression t-test) (**Figure 2.4b**). This is exactly what is expected if VCN licking responses are indeed due to self-generated sounds. Conversely, there was no significant correlation between response to the mimic and response to licking in DCN units (n=13, slope=0.02, $P = 0.79$, $r = 0.0007$, linear regression t-test), such that even DCN units that responded robustly to the mimic, did not respond to licking (**Figure 2.4b**).

One possibility is that the overall sensitivity of DCN units to sound is reduced during licking behavior. Indeed, an overall suppression of auditory responsiveness during behavior has been reported in a variety of systems (Eliades & Wang 2002; Poulet & Hedwig 2003) including mouse auditory cortex (Schneider et al. 2014). To test this we compared DCN unit responses to an external sound (bandpass filtered noise 5-15 kHz, 15 dB SPL) delivered either during licking or when the mouse was still. Evoked responses were indistinguishable under the two conditions (n=9, P=0.49, Wilcoxon Rank Sum Test, **Figure 2.5**). These results are inconsistent with an overall suppression of auditory

sensitivity in DCN during licking and point instead to a mechanism for selectively filtering out self-generated sounds.

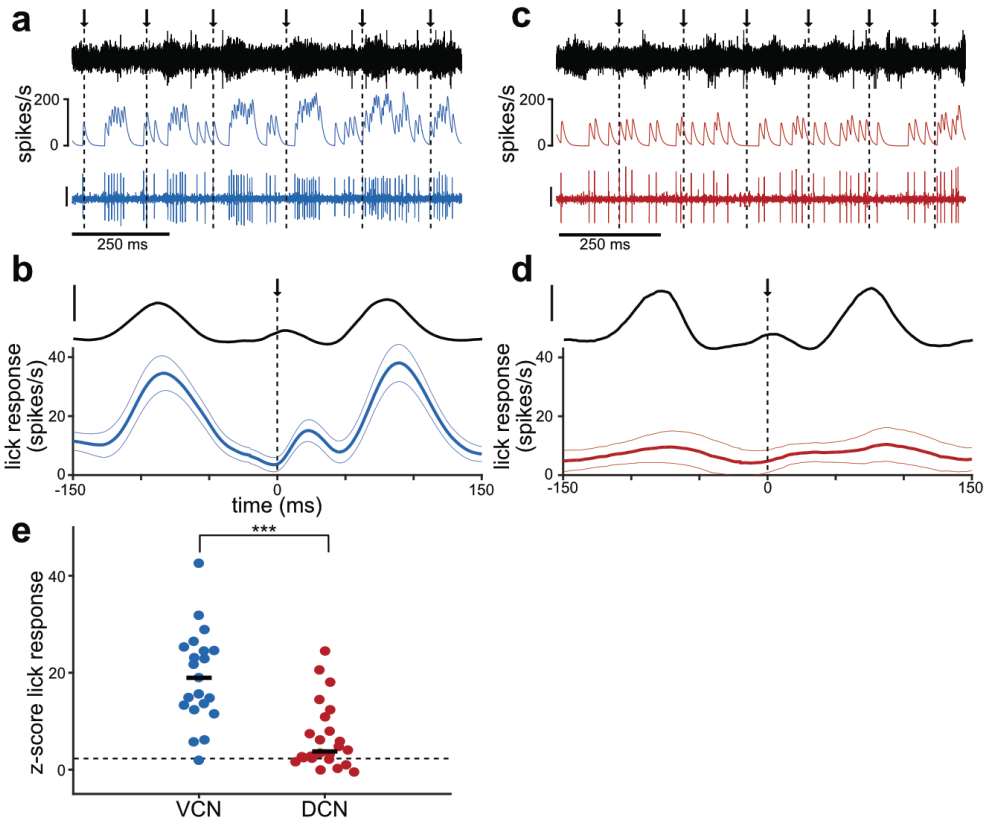

Figure 2.4| Suppression of self-generated sounds in DCN. a, Example VCN unit response during licking. Arrows and dotted lines indicate times of tongue contact with the lick spout. Traces represent the microphone recording (top), smoothed firing rate (middle), and the VCN unit recording (bottom; scale: 30 uV**). b,** Top, average RMS amplitude of the licking sound during VCN unit recordings (scale: 1 a.u.). Bottom, average VCN lick-triggered firing rate (n = 21). Thin lines are s.e.m. **c,** Example DCN unit response during licking. Scale bars and display same as in a. **d,** Bottom, average lick-triggered responses of all DCN units (n = 25), excluding those exhibiting complex-spikes. Compared to VCN units, DCN units exhibited smaller temporal modulations related to licking. Scale bars and display same as in a. **e,** Z-scored lick responses of VCN and DCN cells. Lines indicate median responses. Dashed line indicates z-score > 2.33, alpha = 0.01.

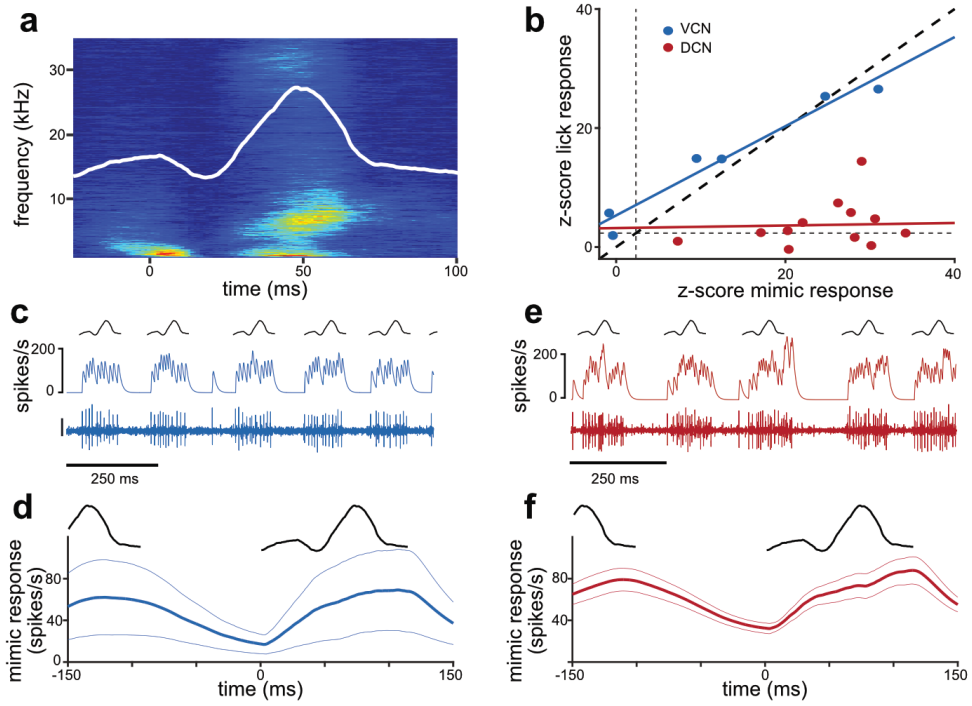

Figure 2.5| Observed suppression of self-generated sounds is not due to differing auditory sensitivities. **a**, Spectrogram of the mimic of the licking sound. **b**, Responses during licking were highly correlated to those observed in the same units during licking for VCN but not DCN recordings. Dotted line shows the unity line. **c**, Example VCN unit response to the mimic. Traces represent the RMS of the mimic (**top**), smoothed firing rate (**middle**), and the VCN unit recording (**bottom**; scale: 30 uV). **d**, **Top**, RMS of the mimic. **Bottom**, average VCN unit response to the mimic (n = 6). Thin lines are s.e.m. **e,f**, Same scale bars and display as **c,d** but for DCN unit responses to the mimic (n = 13). VCN and DCN unit responses to the mimic were not significantly different (P = 0.32, Wilcoxon Rank Sum test).

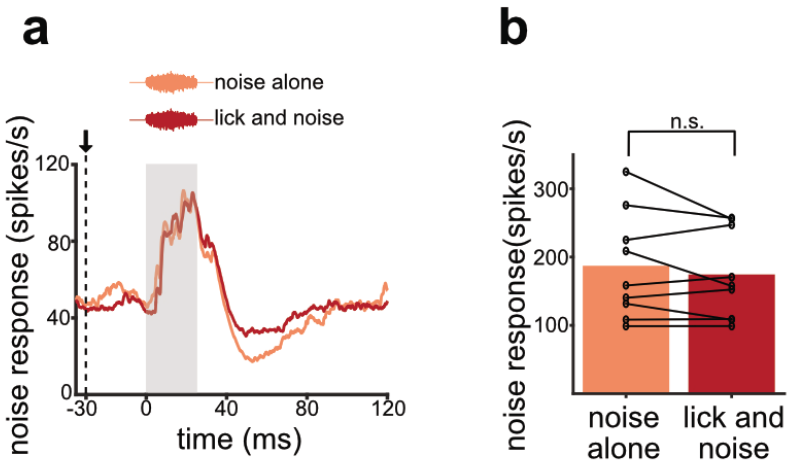

Figure 2.6| Licking does not cause an overall suppression in DCN cells. **a**, example DCN unit response to a noise stimulus (bandpassed filtered 5-15 kHz, 15 dB SPL) played while the mouse was still versus during licking. Gray bar indicates noise duration. **b**, no differences in noise responses were observed when the mouse was still versus licking. n.s. – not significant.

2.3. Discussion

The function of multisensory integration and the cerebellum-like circuitry in the DCN has long been a mystery. One long-standing hypothesis is that the DCN cancels self-generated noise. Previous experiments studying the role of multisensory integration in DCN were performed in either decerebrate or anesthetized preparations using electric stimulation to activate non-auditory inputs to DCN and studied their effects on externally delivered sounds (Kanold et al. 2011; Koehler et al. 2011) and were unable to test responses to self-generated noises. By using an awake preparation, I was able to directly test this hypothesis by observing responses to noises the mouse naturally makes while performing a behavior, namely licking.

I took advantage of the parallel processing of the auditory nerve by comparing responses from units in VCN, a division of the cochlear nucleus that lacks a cerebellum-like structure, to those in the DCN. Though a previous study showed excitatory responses in VCN to electrical stimulation of the trigeminal ganglion in an anesthetized guinea pig (Shore et al. 2003), responses in VCN cells I recorded from faithfully followed the RMS of the self-generated sounds as recorded from a microphone near the animal's mouth, it was concluded that the cells were responding to self-generated sounds, and not non-auditory input related to the animal's licking behavior. This conclusion was further supported by the high correlation between responses to licking and to an externally presented noise that mimicked the self-generated noises the animals made while licking. Responses in VCN cells also showed that not only are the self-generated

lick sounds within the mouse's hearing range, but they may also interfere with the perception of soft external, potentially behaviorally relevant, sounds.

Simple-spiking units recorded in DCN on the other hand had much weaker responses to self-generated noise due to licking. A simple explanation is differences in receptive field or sensitivity, however both DCN and VCN cells showed similar responses to the mimic making this explanation less likely. Another explanation could be an overall reduction of sensitivity in DCN while the animal is performing behaviors. This has been shown to be the case in the cricket auditory system (Poulet & Hedwig 2003; Poulet & Hedwig 2006) and in mouse auditory cortex (Nelson et al. 2013; Schneider et al. 2014). Poulet and Hedwig showed that in crickets, the response of the Omega 1 neuron, a neuron that receives primary afferent input in the auditory system of the cricket, to an external sound was reduced while the cricket was chirping (Poulet & Hedwig 2003). Similarly, responses in mouse auditory cortex to externally delivered tones were reduced while the mouse was performing behavior such as walking or grooming (Schneider et al. 2014). However, since units in DCN respond to sound just as well while licking as while at rest, this explanation is also not the case. Rather the DCN appears to selectively cancel only the self-generated noise while remaining sensitive to external sounds. Similar to cerebellum-like structures in fish.

A few units in DCN showed robust responses to self-generated sounds similar to those found in VCN. One explanation for such a variation is differences in cell-type. Auditory nerve fibers synapse onto both vertical and fusiform cells though only fusiform cells also

receive parallel fiber (and therefore non-auditory) input (Young et al. 1995; Osen 1969a; Hirsch & Oertel 1988; Mugnaini et al. 1980). Therefore cancellation of self-generated noise would only be expected in fusiform cells but not vertical cells. Though unambiguous criteria for linking unit response properties to DCN cell-types have not yet been established for the awake mouse DCN (Ma & Brenowitz 2012), in previous studies, cell-types were determined by their auditory receptive fields, responses to noise, and spontaneous rates (Hancock & Voigt 2002; Rhode 1999; Young & Brownell 1976; Young 1980; K. A. Davis et al. 1996). While future studies would benefit from antidromic stimulation from inferior colliculus (Young 1980), a target for fusiform cell axons, to verify cell type, units recorded in DCN showed high spontaneous rates and excitatory responses to noise indicating that they correspond mainly to output cells.

Cancellation of self-generated sounds at such an early processing stage could provide mammals with a dedicated channel through which unexpected, behaviorally relevant auditory signals are used to rapidly guide behavior such as orienting or escape. This interpretation is consistent with the effects of cutting the dorsal acoustic stria, the output tract of the DCN, in cats, which disrupts orienting to but not discriminating sound source locations (May 2000; Sutherland 1998). Further, it is also consistent with the observations that, in addition to projecting to the inferior colliculus, the DCN also projects directly to auditory thalamus (Malmierca et al. 2002), auditory cortex (Anderson et al. 2009), and areas involved in the acoustic startle response (Lingenhöhl & Friauf 1994).

Sixty years after Von Holst and Mittelstaedt published their findings, Kim et al. (A. J. Kim et al. 2015) observed that neurons in the *Drosophila* visual system received motor corollary discharge inputs with the proper sign, amplitude, and latency to be able to suppress the neurons' responses to the expected visual consequence. What inputs are used to cancel self-generated noise due to licking? This question is the subject of the next chapter.

3. CHAPTER 3: MOSSY FIBER INPUT FROM THE SPINAL TRIGEMINAL NUCLEUS IS NECESSARY FOR CANCELLATION OF SELF-GENERATED SOUNDS

3.1. Introduction

Von Holst and Middelstaedt (Holst & Mittelstaedt 1950)and Roger Sperry (Sperry 1950) theorized that information about animals' behaviors can be used to predict and cancel the sensory consequences of those behaviors. Evidence in support of this hypothesis has been observed in a number of sensory systems in many model organisms (Poulet & Hedwig 2006; Roy & Cullen 2004; Crapse & Sommer 2008). For example, crickets communicate by rubbing their forewings together creating an audible chirp, however their tympanic membrane is located in the forelegs, just a small distance away. The cricket's tympanic membrane and nerve is fully responsive to the sounds of their own chirps, which can be up to 80 dB SPL in amplitude (Poulet & Hedwig 2001). Therefore crickets must be able to modulate the response to their own chirps or face desensitization of their auditory system or confusion of their chirps with external sounds (such as chirps of other crickets) (Poulet & Hedwig 2003). Poulet and Hedwig show that corollary discharge of the closing of the wing (thus generating a chirp) acts to inhibit the Omega 1 neuron, an auditory neuron that receives afferent input, in phase with the cricket's own chirp (Poulet & Hedwig 2003). They further identified the source of the

corollary discharge input to a neuron in the singing central pattern generator area of the cricket (Poulet & Hedwig 2006). When this neuron was hyperpolarized to prevent action potentials, the Omega 1 neuron responded to the cricket's own chirps (Poulet & Hedwig 2006). Therefore, by using predictive information from motor corollary discharge related to singing, crickets are able to modulate the response of their auditory system to their own chirps.

However, corollary discharge need not be the only information used. Animals performing natural behaviors engage multiple streams of information – proprioception, and other sensory signals e.g. visual and vestibular – that relay information about the behavior itself in addition to motor corollary discharge. In the electrosensory system of mormyrid fish, Sawtell and Williams (Sawtell & Williams 2008) studied the effects of passive movements of the tail (where the electric organ is located) on the ability of both electroreceptor afferents and the principal cells of the mormyrid ELL to faithfully encode movement of an object. They found that electroreceptor afferents but not ELL principal cells were greatly affected by tail movement. They further showed that proprioceptive information of tail position relayed via mossy fibers, was used to oppose the electrosensory consequences of those movements.

In the previous chapter, I showed that simple-spiking cells in DCN have much weaker responses to self-generated licking sounds than those in VCN even though DCN cells responded similarly to a mimic of the licking sound. What inputs mediate these weaker responses? Does licking behavior engage the mossy fiber-parallel fiber system of

DCN? In this chapter, I will first show that licking behavior engages non-auditory input conveyed via the mossy-fiber parallel fiber system to DCN by recording from complex-spiking units in deafened mice. Complex-spiking units correspond to a class of DCN interneuron known as cartwheel cells. They are easily identified electrophysiologically by the presence of two different spike types, a complex spike and a simple spike, a property not found in other cell types of the DCN (Zhang & Oertel 1993; Manis et al. 1994; Golding & Oertel 1997; Portfors & P. D. Roberts 2007; Ma & Brenowitz 2012). Since cartwheel cells only receive input from parallel fibers and other cartwheel cells (Hirsch & Oertel 1988; Zhang & Oertel 1993; Manis et al. 1994; K. A. Davis & Young 1997), response to licking in deafened mice must be due to activation of the mossy fiber-parallel fiber system. Where could such mossy fibers come from?

Anatomical and electrophysiological experiments have shown numerous mossy fiber inputs from the spinal trigeminal nucleus (sp5) to the granule cell domain of the DCN in many animals including the cat, guinea pig, and rat (Itoh et al. 1987; Haenggeli & Pongstaporn 2005; Zhou & Shore 2004; Young et al. 1995; Shore 2005b). Further studies have shown that sp5 receives proprioceptive information from intra-oral structures, the tempro-mandibular joint, and tongue muscles – all structures that are involved in licking (Romfh et al. 1979; Nazruddin et al. 1989; Takemura et al. 1991; Suemune et al. 1992). Such information could be used to predict self-generated noise due to licking behavior. Since mossy fiber input from sp5 has yet to be observed in mice, in this chapter I will confirm the presence of sp5 mossy fiber input in mice by injecting an anterograde viral tracer (AAV2-GFP) into sp5 and looking for terminals in DCN. I will

then determine if these inputs are used to suppress responses in DCN to self-generated noise due to licking by injecting the action potential blocker lidocaine into sp5 while recording from simple spiking cells in DCN. These experiments provide the first evidence for a role for non-auditory, mossy fiber input to DCN in the prediction and cancellation of self-generated sounds.

3.2. **Results**

3.2.1. *DCN granule cells convey non-auditory information related to licking behavior*

In addition to auditory nerve input, DCN receives non-auditory, behavior-related signals conveyed by mossy fibers that could, in principle, underlie a selective cancellation of self-generated sounds. Though electrophysiological correlates of non-auditory, somatosensory mossy fiber inputs to DCN have been characterized using electrical or manual stimulation in anesthetized or decerebrate preparations (Kanold 2001; Shore & Zhou 2006), responses to non-auditory inputs have never been observed in awake, behaving animals. To isolate non-auditory responses, we deafened mice bilaterally by puncturing the tympanic membrane, removing the ossicles, and carefully injecting kanamycin, an antibiotic that kills hair cells of the cochlea, into the oval window (Zettel et al. 2001). Prior to deafening, robust auditory evoked field potentials were seen in DCN to broadband noise. Field potentials had a threshold of approximately

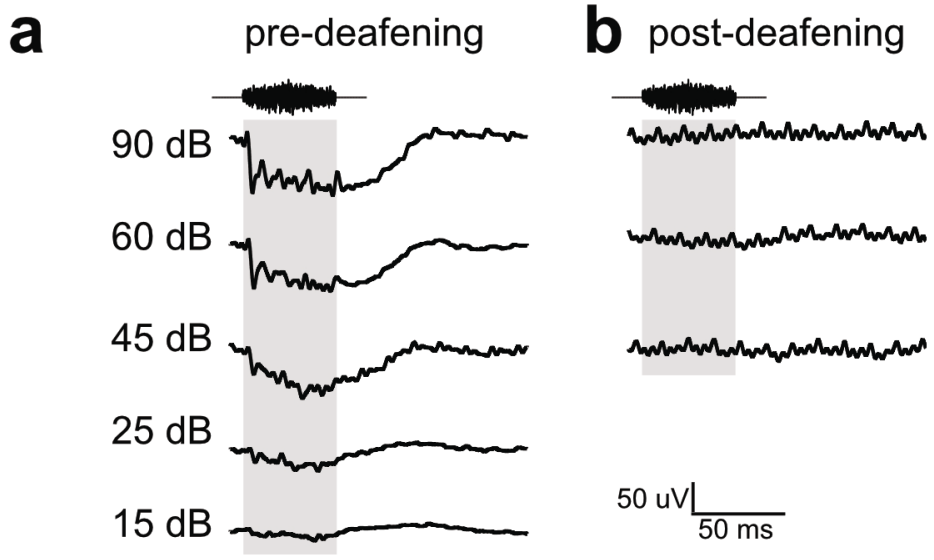

Figure 3.1| Injection of kanamycin into the oval window abolishes sound evoked field potentials in DCN. **a**,**b** Sound-evoked field potentials (50 ms, broadband noise) recorded in DCN of the same mouse before (**a**) and after (**b**) surgical deafening. Note complete absence of sound-evoked responses after deafening.

15 dB SPL (**Figure 3.1a**). After bilateral deafening, auditory field potentials were completely abolished up to 90 dB SPL (**Figure 3.1b**), the upper limit of the experimental apparatus. Further, deafened animals showed no walking or postural abnormalities when in their home cage demonstrating little to no vestibular damage (data not shown).

Complex spikes were defined as high frequency bursts (with inter-spike interval less than 3.5 ms) with progressively decreasing spike heights (Manis et al. 1994; Portfors & P. D. Roberts 2007; Ma & Brenowitz 2012). Complex spikes, and therefore cartwheel cells, were easily identified visually in our extracellular recordings (**Figure 3.2**). For these analyses, complex spikes and simple spikes were considered separately and a complex spike burst was considered one complex spike regardless of number of spikelets contained within the burst. Z-scores of lick responses were computed as described in Chapter 2. 11 complex spiking units were isolated from 3 surgically deafened mice. 9 of 11 cartwheel cells showed significant simple spike responses to licking (z-scores >2.33, α=0.01) while 3 of 11 cartwheel cells showed significant complex spike response **(**example cells: **Figure 3.2b,c,** summary **Figure 3.3a**). Those cells that showed significant complex-spiking responses also showed significant simple-spiking responses. Further, the overall rate of complex-spike firing also increased during licking (n = 11, 1.72 Hz ± 1.63 Hz not licking, 2.27 Hz ± 1.30 Hz licking, mean ± standard deviation, P =0.04, Wilcoxon Sign Rank Test, **Figure 3.3b**). These results suggest that DCN granule cells convey non-auditory information related to licking behavior.

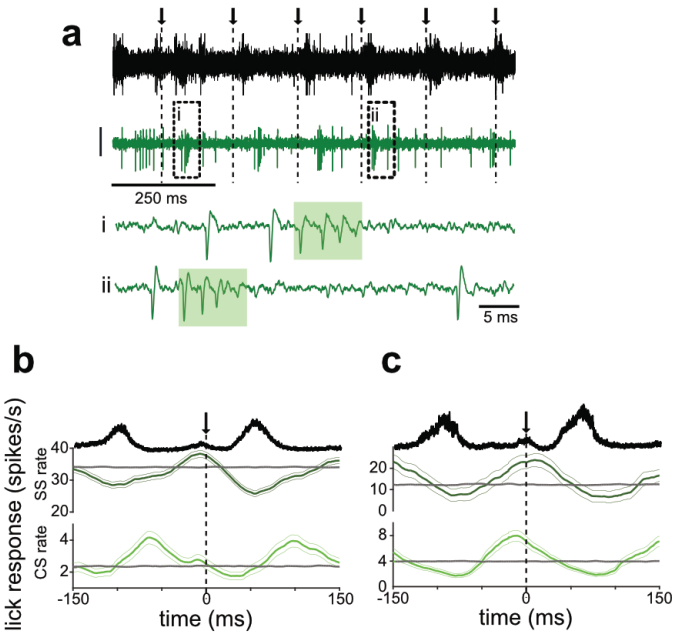

Figure 3.2| Example responses of cartwheel cells to licking in deafened mice. a, Example DCN complex-spiking unit recorded during licking in a surgically deafened mouse. Arrows and dotted lines indicate times of tongue contact with the lick spout. **Top trace**, microphone recording. **Middle trace**, extracellular voltage from a DCN complex-spiking unit (scale: 30 uV). **i, ii,** Expanded traces from boxed regions showing complex-spike (CS) (shaded rectangle) and simple spike (SS) waveforms. **b,c**, Lick-triggered SS and CS firing rates for two complex-spiking units recorded in deafened mice. Thin lines are s.e.m. Gray lines show the average lick-triggered response of shuffled spike trains. Data in **b** are from same unit as example traces in **a**. **Top trace** (black) is the RMS amplitude of the licking sound.

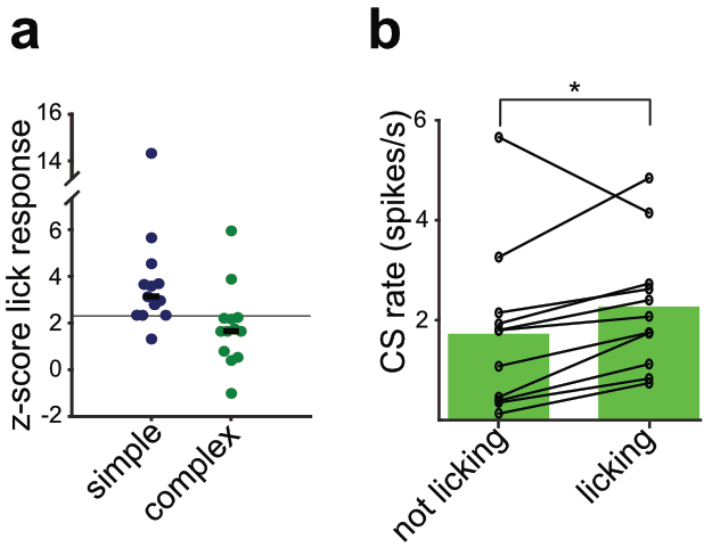

Figure 3.3| Summary of cartwheel cell responses to licking. **a**, Summary of z-scored lick responses recorded in 11 complex-spiking units recorded in 3 surgically deafened mice. 9 showed significant lick responses in their SS firing and 3 showed significant lick responses in their CS firing (z-score > 2.33, alpha=.01). **b**, Overall complex-spike firing rates increased during periods of licking in deafened mice (n = 11, P =0.04, Wilcoxon Rank Sum test).

3.2.2. *Spinal trigeminal input is integral to cancellation of self-generated noise due to licking*

Based on previous anatomical studies of mossy fiber inputs to DCN in other mammals, the spinal trigeminal nucleus (sp5) is a likely source of somatosensory information related to licking (Itoh et al. 1987; Haenggeli & Pongstaporn 2005). Indeed, Haenggeli et al. showed that the DCN receives mossy fiber input from throughout the rostral-caudal extent of sp5. Injection of an anterograde viral tracer (AAV2-GFP) into mouse sp5 resulted in labeled mossy fibers in the granule cell domains of DCN (**Figure 3.4a**). Mossy fiber endings in DCN resembled mossy fibers also labeled in cerebellum (**Figure 3.4b**).

If non-auditory, licking related signals from sp5 serve to selectively cancel self-generated noise due to licking, transiently silencing such inputs should reveal responses during licking in DCN units similar to those observed in VCN units. Indeed, micropressure injections of lidocaine, an action potential blocker, into sp5 led to an increase in the overall firing rate during licking (n=10, 18.26 Hz ± 7.08 Hz, mean ± standard deviation) as well as increased temporal modulation of firing that was correlated with the amplitude of the licking sound (peak-to-trough firing rates, n=10, 11.1 Hz ± 5.0 Hz pre-lidocaine injection, 24.7 Hz ± 18.9 Hz post-lidocaine injection, P=0.002, Wilcoxon Sign Rank Test, **Figure 3.5a**). No such changes were seen in control mice in which saline was injected instead of lidocaine (peak-to-trough firing rates, n=8, 12.3 Hz ± 7.3 Hz pre-saline injection, 13.2 Hz ± 7.9 Hz post-saline injection, P=0.46, Wilcoxon

Sign Rank Test, **Figure 3.5b**). There was no difference in pre-injection responses between the lidocaine and saline groups (P=0.57, Wilcoxon Rank Sum Test). For further analysis, responses to licking were converted to z-scores as in Chapter 2. Injection of lidocaine resulted in an increase in z-scored lick responses (P=0.0098, Wilcoxon Sign Rank Test, **Figure 3.6a**) while injections of saline resulted in no changes in z-scores (P=.31, Wilcoxon Sign Rank Test **Figure 3.6a**). Since z-scored responses to the mimic were the same in both groups (8.20 ± 3.47 for lidocaine group, 8.76 ± 3.54 for saline group, P=0.87, Wilcoxon Rank Sum Test), differences in the two groups cannot be explained by differing auditory sensitivities (**Figure 3.6b**). Further, injection of lidocaine or saline had no effect on lick rate (P=0.77, Wilcoxon Signed Rank Test for lidocaine, P=0.25, Wilcoxon Sign Rank Test for saline, **Figure 3.6c**). Changes in RMS amplitude of the lick sound over the course of licking did not differ between the lidocaine and saline groups (P=0.51, Wilcoxon Rank Sum Test, **Figure 3.6d**) and changes in RMS amplitude of the lick sound do not correlate with changes in z-score lick responses (**Figure 3.6d**).

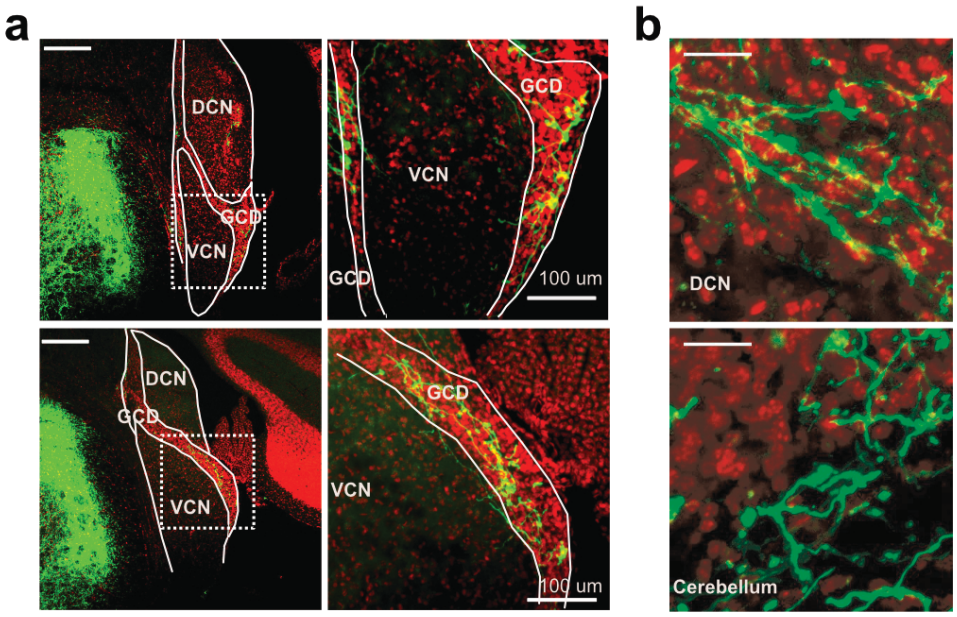

Figure 3.4| Anterograde tracing from the spinal trigeminal nucleus labels mossy fibers in the granule cell domain of DCN **a,** Labeled mossy fibers were observed in DCN granule cell domains (GCD) after injection of an anterograde viral tracer (AAV2-GFP) into the ipsilateral spinal trigeminal nucleus (Sp5). Scale bar: 200 um. *Right*, higher magnification views of areas indicated by dotted rectangles. Scale bar: 100 um. **b,** Similar appearance of Sp5 mossy fiber terminals labeled in the granule cell domains of DCN (top) and in the cerebellar granular layer (bottom). Scale bars: 50 um.

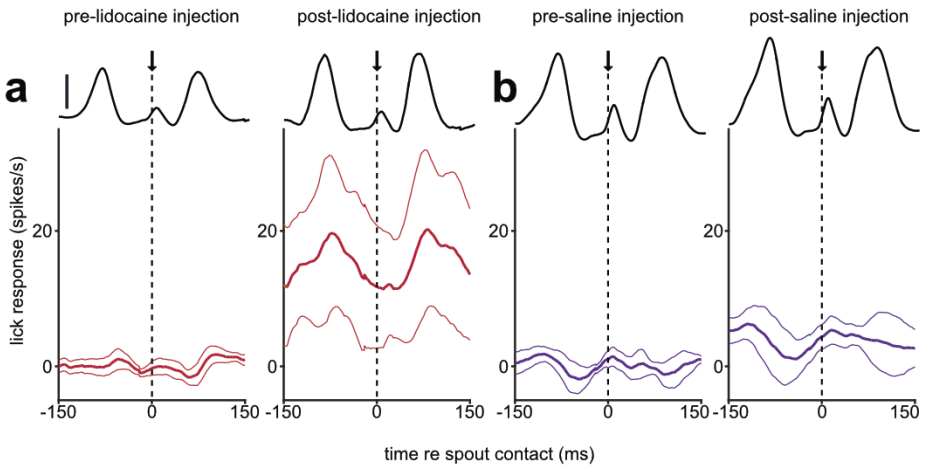

Figure 3.5| Injection of lidocaine into spinal trigeminal nucleus uncovers responses to self-generated sounds due to licking. **a,b**, Lick-triggered response of DCN cells before (left) and after (right) micropressure injection of lidocaine (panel **a,** $n = 10$) or saline (panel **b,** $n = 8$) into Sp5. DCN cells showed responses to licking after injection of lidocaine and these followed the RMS of the microphone. Thin lines are s.e.m. Solid black lines show the RMS amplitude of the licking sound (scale bar: 1 a.u.).

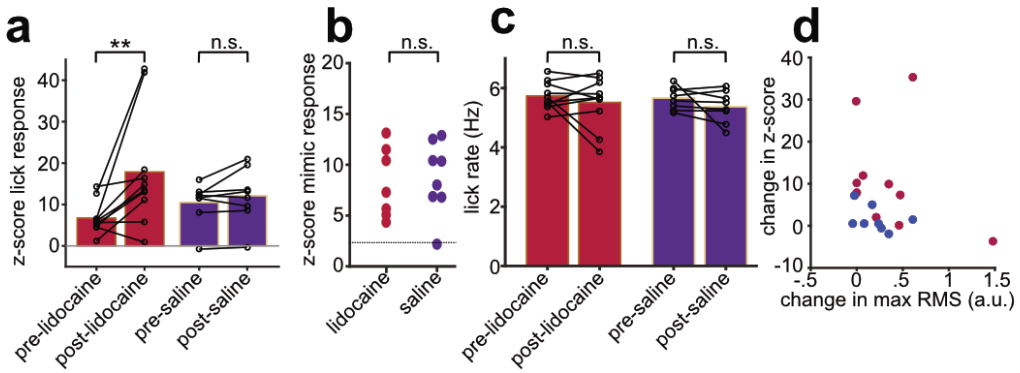

Figure 3.6 | Injection of lidocaine into spinal trigeminal nucleus uncovers responses to self-generated sounds due to licking. **a**, Lidocaine injection resulted in a significant increase in z-scored lick responses in DCN units **b**, Auditory responses to the mimic were not significantly different in lidocaine and saline groups. **c,** Lick rate did not differ before and after injection of lidocaine or saline. **d,** Changes in z-score lick responses were not correlated with changes in the RMS amplitude of the licking sound after lidocaine. Changes in RMS amplitude of the licking sound did not differ between lidocaine and saline groups.

3.3. **Discussion**

Previous studies have found electrophysiological evidence for non-auditory input to DCN in anesthetized or decerebrate preparations by tugging the external ear (Kanold 2001) or electrical stimulation of the dorsal column nuclei and spinal trigeminal nucleus(Young et al. 1995; Shore et al. 2008; Saade et al. 1989; Shore 2005a) but none have shown non-auditory responses in DCN during natural behaviors. By recording in cartwheel cells in an awake, deafened mouse preparation, I was able to isolate responses to non-auditory input due to behavior. Since the only known inputs to cartwheel cells are granule cells and other cartwheel cells, the temporal modulations in simple and/or complex-spike firing rates to licking suggest that the DCN granule cells convey non-auditory information related to licking behavior.

While previous studies in cat, guinea pig, and rat have shown evidence for inputs from sp5 both anatomically and electrophysiologically (Itoh et al. 1987; Young et al. 1995; Shore 2005a; Haenggeli & Pongstaporn 2005), no such studies have been performed in mouse. The presence of mossy fiber endings in the granule cell domains of DCN after injection of AAV2-GFP into sp5 are congruent with previous anatomic studies done in guinea pig and rat (Itoh et al. 1987; Haenggeli & Pongstaporn 2005) and suggests that sources of mossy fiber input to the granule cell domain of DCN might be conserved across species. Further retrograde studies from the DCN to determine other mossy fiber sources would enhance our understanding of potential non-auditory inputs to mouse DCN and would reveal similarities or differences in DCN mossy fiber input across species.

Studies in many sensory systems including those performed in the cricket auditory system (Poulet & Hedwig 2003; Poulet & Hedwig 2007), and the electrosensory system of weakly electric fish (Sawtell & Williams 2008) have provided evidence for information related to movement being used to cancel self-generated sensory signals related to those movements. Chapter 2 showed that simple-spiking cells in DCN had weak responses to self-generated noise. The increase in temporal responses to licking after injection of lidocaine into sp5 provides evidence for sp5 playing a role in the cancellation of self-generated licking noise. These increases could not be explained by changes in licking behavior or changes in the sound of licking. While some units showed a large increase in z-score lick response, other units showed a more modest increase. Since mossy fiber input to DCN comes from throughout the rostral-caudal extent of sp5 (Haenggeli & Pongstaporn 2005), these differences could be due to incomplete inactivation of mossy fibers.

Sp5 is not the only source of non-auditory input to DCN. Anatomic and electrophysiological evidence for other sources of mossy fiber input include the dorsal column nuclei (Itoh et al. 1987; Weinberg & Rustioni 1987; Saade et al. 1989; Young et al. 1995), the dorsal root ganglion of C2 (Kanold 2001), the pontine nuclei (Ohlrogge et al. 2001), lateral reticular formation (Zhan & D. K. Ryugo 2007) and vestibular nerve (Burian et al. 1989; Wigderson et al. 2016) suggesting that the DCN's ability to cancel self-generated noise is not restricted to noises from licking behavior. Indeed a recent study in the rat (Wigderson et al. 2016) found that a subset of cells in the DCN respond differentially to rotation of the rat versus rotation of the speaker, though the auditory

stimulation was the same in both cases suggesting that the DCN can discriminate between motion of the body versus motion of a external sound source. The DCN might be able to cancel self-generated sounds related to a wide variety of behaviors including movement of the external ears, stepping, and movement of the head that would aid to process external behaviorally relevant sounds more effectively.

4. CHAPTER 4: THE DORSAL COCHLEAR NUCLEUS IS AN ADAPTIVE FILTER FOR SELF-GENERATED SOUNDS

4.1. Introduction

The previous chapters have shown that the DCN suppresses responses to self-generated noise during licking and that that this suppression is mediated by mossy fiber input via sp5. Experiments performed in mouse brain slices have shown that parallel fiber synapses onto both cartwheel cells and fusiform cells are plastic. Tzounopoulos et al. (Tzounopoulos et al. 2004) showed in mouse brain slices that repetitions of an EPSP in cartwheel cells caused by stimulation of parallel fibers followed 5 ms later by a spike caused a decrease in amplitude of the EPSPs, while no change occurred in EPSP amplitude if spikes preceded EPSPs (an anti-Hebbian learning rule). Interestingly, the same pairing protocol caused increases in amplitude of EPSPs (a Hebbian learning rule) in fusiform cells. However, when the olivocochlear bundle, a cholinergic input to DCN, was stimulated, the learning rule at parallel fiber synapses onto fusiform cells switched and followed an anti-Hebbian learning rule similar to that seen in cartwheel cells. (Zhao & Tzounopoulos 2011). What function could such plasticity perform *in vivo*?

Studies in similar cerebellum-like sensory structures found in the electrosensory system of mormyrids, gymnotids, and elasmobranches with similar plasticity rules at

their parallel fiber synapses provide some clues. Mormyrids have both a passive electrosensory system that detects weak external electric fields, e.g. those generated by prey, and an active electrosensory system in which an electric organ, a modified muscle, emits a high frequency electric pulse known as an electric organ discharge (EOD). The active electrosensory system is able to detect nearby objects by distortions objects cause in the self-generated electric field. These distortions are detected by a separate set of electroreceptors specially tuned to the high frequency of the EOD. However, passive electroreceptors also respond to this discharge as well (Bell & C. J. Russell 1978) therefore the electrosensory system must filter out the consequences of the EOD on the passive electroreceptors. The electrosensory system does this in the ELL. Principal cells in the ELL receive two types of inputs: primary electrosensory inputs from afferents, and parallel fibers which convey a diverse set of sensory and motor signals including corollary discharge related to the EOD motor command. These corollary discharge signals conveyed via parallel fibers are used to cancel the predictable sensory consequences of the EOD (Bell 1981).

Since the sensory consequences of the EOD changes as a function of the conductivity of the water (Bell & C. J. Russell 1978), the ELL must also be able to adjust to these changes. In 1981, Bell (Bell 1981) showed the ELL could adaptively filter sensory consequences of the EOD by performing experiments in a paralyzed preparation that blocked the EOD, but preserved EOD motor commands. Principal cells of the ELL had little to no response to the EOD command alone. When he paired an external electrosensory stimulus with the EOD command, the response to the stimulus decreased

over time (Bell 1981), and when the stimulus was turned off, the cells responded to the EOD command alone in a manner that was opposite that of the initial response, termed a "negative image." Intracellular recordings *in vivo* show the principal cells of the ELL as the site of negative image formation (Bell et al. 1993). Further experiments in ELL slice preparations showed that parallel fiber inputs that preceded a postsynaptic spike were subsequently depressed while parallel fiber inputs at other delays were strengthened, an anti-Hebbian learning rule (Bell, Han, et al. 1997). Further modeling studies showed that these plasticity rules observed in *in vitro* preparations could be responsible for negative images shown previously in *in vivo* experiments (P. D. Roberts & Bell 2000).

More recent studies have shown that negative image formation is not restricted to pairings with the EOD command. Requarth and Sawtell showed that pairing with corollary discharge signals relating to the movement of the tail and trunk also can be used to form negative images (Requarth & Sawtell 2014). Further, pairings with passive displacements of the tail also formed negative images showing that proprioceptive information is used by the ELL to cancel sensory consequences of tail position (Sawtell & Williams 2008; Requarth et al. 2014).

Similar to the passive electrosensory system of mormyrids, electroreceptors of the elasmobranch electrosensory system sense weak electric fields occurring in the water. However, movements related to the fish's breathing can modulate the electroreceptors through half their dynamic range (MONTGOMERY 1984). Interestingly, such modulation is absent in the principal neurons (called AENs) of the dorsal octavolateral

nucleus (DON), a cerebellum-like structure that receives primary electrosensory afferent input (MONTGOMERY 1984; Bodznick et al. 1992). When an electrosensory stimulus is paired with proprioceptive or motor information related to ventilation, responses to that stimulus decreases over time, and when the stimulus is removed, negative images similar to those found in ELL are observed (Bodznick 1993).

Not only are the ELL and DON able to cancel sensory consequences due to the animal's behavior but they are also able to update their predictions of the sensory consequences. This ability is due to anti-Hebbian plasticity found at parallel fiber synapses onto principal cells. In this chapter, I will show that DCN responses to noises that are played correlated to licking behavior are reduced over time. These results provide the first evidence that the DCN, like the ELL and DON, adaptively cancels the sensory consequences of behavior.

4.2. **Results**

To test whether DCN units are capable of adaptive filtering we delivered an external sound (bandpassed noise 5-15 kHz, 15dB SPL) temporally correlated with licking (30 ms after tongue contact). Responses to sound were defined as the peak-to-trough firing rate of the average sound response of 150 sound presentations. We observed that DCN unit responses to the correlated sound gradually declined over the course of several minutes of continuous licking (~1000 paired lick-sound presentations). Such declines ranged from modest reductions with the peak-to-trough noise response

falling to 72% of its initial value (**Figure 4.1a**), to nearly complete cancellation of the correlated sound with the noise response falling to 34% of its initial response (**Figure 4.1b**). 7 of 10 DCN units showed significant reductions (α=0.01, linear regression t-test). Overall, DCN unit responses decreased when noise presentations were correlated with licking (n=10, P=3.2 x 10^{-11}, linear regression t-test; **Figure 4.2,** red line). On average, responses to correlated sounds decreased to 76% ± 17% of the initial sound evoked response. Since, there was no change in lick rates over the course of pairing (P=0.75, Wilcoxon Sign Rank Test), these changes cannot be explained due to changes in licking behavior.

One explanation for these decreases is adaptation of the peripheral auditory system. However, when an identical sound is played uncorrelated to licking, 5 of 7 DCN cells showed increase in responses (α=0.01, linear regression t-test). Overall, unit responses showed increases to uncorrelated sound presentations (n=7, P=0.013, linear regression t-test, example cells: **Figure 4.1b,c,** summary: **Figure 4.2** blue line). Further, VCN unit responses exhibited no change when sound presentations were correlated with licking (n=4, P=0.27, linear regression t-test, example cell: **Figure 4.1d**, summary: **Figure 4.2** yellow line). Taken together, this makes adaptation at the level of the auditory nerve an unlikely explanation.

We performed preliminary tests on the temporal specificity of the suppressed responses to the correlated sound by repeatedly pairing the above sound with licking at one of two fixed delays, 15 ms or 50 ms, relative to tongue contact. After extended

pairing, (2-3 sessions, 6000-9000 licks), sound-evoked responses were briefly probed at each delay. On average, the ratio of the sound-evoked responses at the paired delay to the sound-evoked responses at the unpaired delay was 0.72 ± 0.086. This reduction in response was significant (n=6 units, P=.031, Wilcoxon Sign Rank Test, **Figure 4.3**). These observations are consistent with the idea that DCN generates a cancellation signal that is specific to phase or timing within the lick cycle. Pairing induced changes in the strength of auditory inputs cannot explain these results because the auditory stimulus is identical in the two probe conditions, suggesting that the cancellation signal is due to changes in the strength of licking-related signals conveyed by granule cells.

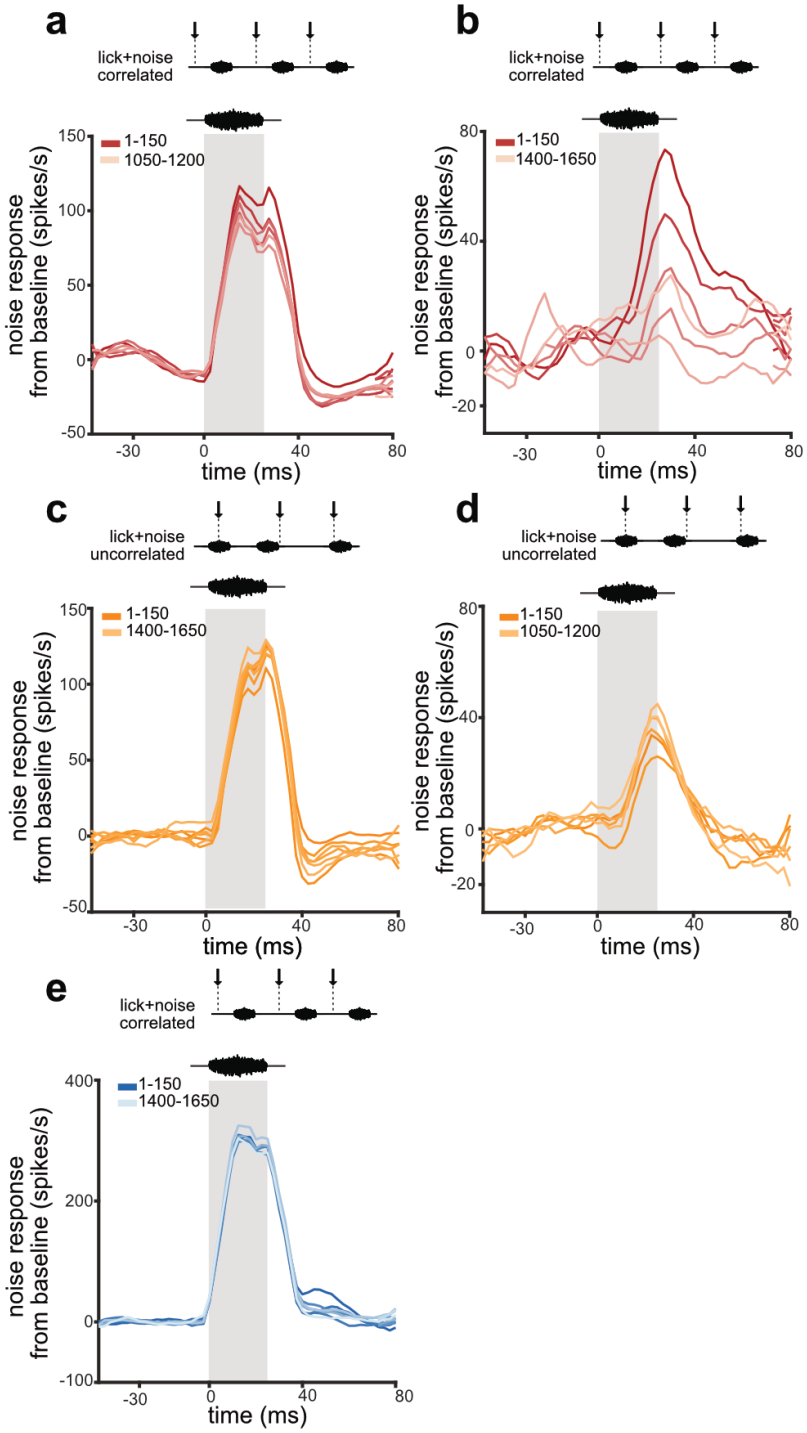
a
lick+noise correlated
1-150
1050-1200
noise response from baseline (spikes/s)
time (ms)
b
lick+noise correlated
1-150
1400-1650
noise response from baseline (spikes/s)
time (ms)
c
lick+noise uncorrelated
1-150
1400-1650
noise response from baseline (spikes/s)
time (ms)
d
lick+noise uncorrelated
1-150
1050-1200
noise response from baseline (spikes/s)
time (ms)
e
lick+noise correlated
1-150
1400-1650
noise response from baseline (spikes/s)
time (ms)

Figure 4.1 External sounds correlated with licking are suppressed in DCN. **a-d,** Sound-evoked responses for example DCN units in which noise (5-15 kHz, 15 dB SPL) was played either correlated (**a**,**b**) or uncorrelated (**c**,**d**) with licking (schematized above each plot with spout contact indicated by arrows and dotted lines). Each line is the mean sound-evoked response averaged over 150 noise presentations with lighter colors representing progressively later sound presentations. Solid gray bar indicates sound duration. **e,** Same as **a**,**b** except for an example VCN unit in which noise was correlated with licking.

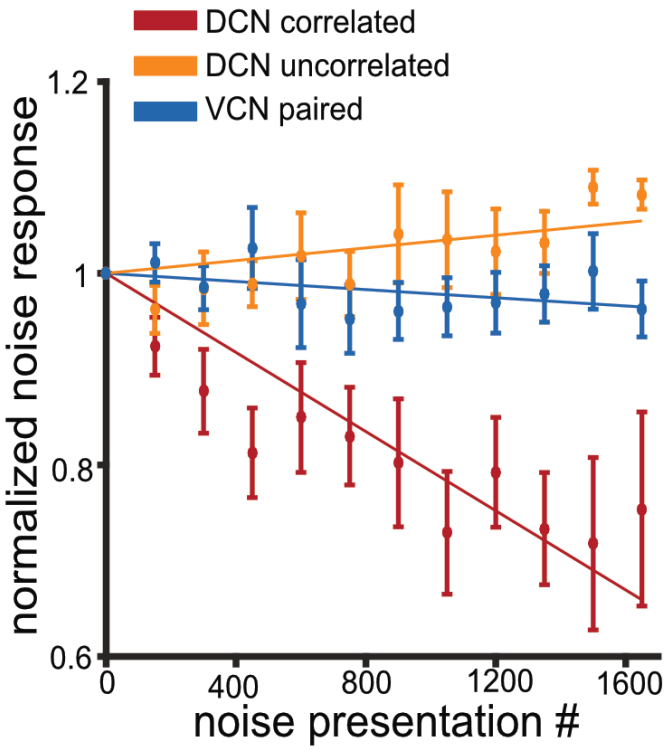

Figure 4.2 | Group data showing changes in sound-evoked responses over the course of repeated sound presentations. Overall, DCN unit responses decreased when sound presentations were correlated with licking (n = 10, P = 3.2 x 10^{-11}, linear regression t-test) and slightly increased when sound presentations were uncorrelated with licking (n = 7, p = 0.013, linear regression t-test). VCN unit responses exhibited no change when sound presentations were correlated with licking (n = 4, P = 0.27, linear regression t-test).

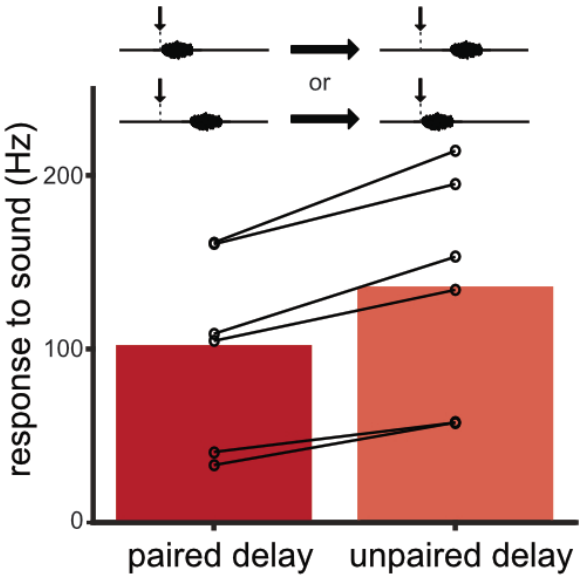

Figure 4.3| Suppression of correlated sounds to licking is temporally specific. Sounds were played correlated to licking at one of two delays relative to tongue contact with the water spout and then probed at the opposite delay (schematized above bar plot). DCN units had lower responses when the sound was played at the paired delay compared to when the sound was played at the unpaired delay.

4.3. **Discussion**

Here I show for the first time the DCN adaptively suppresses sounds played correlated to licking behavior. Such an ability would be important for suppressing self-generates sounds when the auditory consequences of a given behavior change. For example licking foods of different textures or walking on dry versus wet sand could produce different self-generated sounds though the animal is performing the same behavior. Being able to adapt to these changes allows the animal to better process external sounds in a changing environment. Since similar decreases in noise response were not seen in VCN cells or in DCN cells when sound was played uncorrelated with licking, observed decreases are not due simply to adaptation of auditory nerve responses.

Another possible explanation plasticity at auditory nerve fiber synapses. However, studies in mouse brain slice preparations found no evidence for plasticity at auditory nerve fiber synapses onto fusiform cells. When EPSPs evoked by stimulation of parallel fibers were paired with an intracellular current evoked spike, subsequent stimulation of parallel fibers caused smaller EPSPs in cartwheel cells (Tzounopoulos et al. 2004) and larger and smaller (after activation of cholinergic inputs) in fusiform cells (Tzounopoulos et al. 2004; Zhao & Tzounopoulos 2011). However similar pairing protocols in which EPSPs were evoked by auditory nerve stimulation caused no changes in amplitude of auditory nerve evoked EPSPs after pairing (Tzounopoulos et al. 2004; Zhao & Tzounopoulos 2011). Never the less, Koehler et al. (Koehler & Shore 2013) observed that after pairing electrical stimulation of sp5 with tones, DCN unit responses to

tones presented in the absent of sp5 stimulation both increased and decreased. Such an effect could be due to plasticity at auditory synapses. However, results in this chapter suggest that suppression of correlated sounds was temporally. After pairing, when sound was played at a different delay, sound-evoked responses were larger than when sound was played at the paired delay. Since plasticity at auditory nerve fiber synapses would affect sound-evoked responses in both conditions, this is not a likely explanation. Differences in results presented in this chapter from those of Koehler et al. could be due to differences in anesthetic state of the animal or electrical stimulation of sp5. Using an awake, behaving preparation allowed for more natural engagement of the mossy fiber-parallel fiber system.

What could be a possible mechanism for sensory cancellation in DCN? Clues from cerebellum-like structures in the electrosensory system of fish may provide insights. Cancellation of sensory consequences of behavior also occurs in the ELL of weakly electric mormyrid fish where a "negative image" of the predictable sensory consequence is formed via plasticity at parallel fiber synapses (Bell 1981). These negative images cancel the effecst of self-generated electrosensory stimuli. Further both electrophysiological and modeling studies have linked anti-Hebbian plasticity at parallel fiber synapses onto output cells to generation of negative images (Bell, Han, et al. 1997; P. D. Roberts & Bell 2000; Bell et al. 1993). Similar anti-Hebbian plasticity has been shown in parallel fiber synapses onto both cartwheel cells and fusiform cells (Tzounopoulos et al. 2004; Zhao & Tzounopoulos 2011). However, observing negative images in this study was difficult for two reasons. First, keeping well-isolated units over

a long enough period of time during licking to both present correlated sound stimuli and to probe for negative images by removing the stimulus was challenging. Second, even when units remained well-isolated, mice often became satiated and stopped licking prior to being able to probe for formation of a negative image. As a result, we cannot yet say whether cancellation observed in DCN is due to the generation and subtraction of negative images.

Future experiments could be able to remedy the above difficulties using an optogenetic approach. Injecting an adeno-associated viral (AAV) vector containing channel rhodopsin into sp5 would cause channel rhodopsin to be expressed in mossy fibers of DCN. Light activation of mossy fibers could then be paired with auditory stimuli in an awake, but still (i.e. not behaving) mouse. This would make holding single units easier and allow for many more lick-sound pairings and the ability to probe for negative images. A disadvantage of this experiment is that potentially, only a subset of mossy fibers would be activated while a natural behavior would activate a full complement of mossy fibers associated with it.

Even so, negative images may still not be observed. Since cartwheel cells do respond to sounds (Portfors & P. D. Roberts 2007; Ma & Brenowitz 2012), including the mimic of the lick sound (**Appendix A**), and they only receive parallel fiber input, auditory information is itself transmitted via parallel fibers. The paired sound then may be integral in activating the parallel fiber input to generate the negative image. If this were the case, negative images would not be observed in output cells by simply removing

the paired sound and observing responses to licking behavior alone, as they are observed in the ELL of fish by removal of the external paired electrical stimulus and observing responses to corollary discharge signals of the EOD alone (Bell 1981).

Another question is why the degree of cancellation differed across cells (**Figure 4.1a,b**). This could be due to multiple reasons. While care was taken to place the speaker at eye level with the mouse and center it at the mouse's midline, constraints in the experimental apparatus led the speaker to be slightly to the right of the midline. Since the DCN also receives input from the cochlear nuclei at the opposite side, differences in binaural cues could reduce the degree of sensory cancellation. Future studies using speakers directly coupled to the ears would minimize such effects. Further, correlating sound stimuli with a fixed delay from tongue-to-spout contact may have caused too much variation in sound presentation relative to licking behavior. Tongue-to-spout contact is a simple but crude measure of licking behavior, which can vary trial to trial. Pairing the same sound at a fixed delay from tongue-to-spout contact does not take this variation into account. Therefore the relationship between parallel fiber input from behavior and its auditory consequence also varies. A conceptually more appealing experiment would be to directly correlate amplitude modulation of continuous noise with jaw movement. Licks with smaller jaw movements would have smaller amplitude modulation while licks with larger jaw movements would have larger amplitude modulation. This would make for a more consistent auditory consequence of behavior and perhaps cause larger suppression effects.

5. CHAPTER 5: DISCUSSION AND FUTURE DIRECTIONS

5.1. **Discussion**

This thesis demonstrated that a cerebellum-like structure in the auditory system of the mouse, the dorsal cochlear nucleus (DCN), is able to adaptively cancel self-generated sounds. In Chapter 2, I found that the DCN but not the ventral cochlear nucleus (VCN), is able to suppress responses to self-generated sounds due to licking. Importantly, the suppression did not affect responses to external sounds played during licking showing that sensory cancellation was not simply due to an overall reduction in sensitivity to sound during licking. Experiments performed in Chapter 3 set out to determine if non-auditory mossy fiber inputs mediate the cancellation observed in Chapter 2. Indeed, recordings from cartwheel cells in deafened mice revealed that non-auditory, mossy fiber-parallel fiber system is engaged while the animal is licking. Further, silencing mossy fiber inputs from sp5 using lidocaine revealed licking related responses in DCN neurons. These responses were similar to those observed in VCN and likely reflect responses to self-generated sounds related to licking. Together, these results suggest that mossy fiber inputs to DCN from sp5 function to cancel out self-generated sounds. In Chapter 4, I found that sensory cancellation in DCN is plastic, similar to that described previously in cerebellum-like structures in fish. DCN neuron responses to acoustic stimuli delivered correlated to licking decreased while responses to acoustic simuli

delivered uncorrelated to licking slightly increased. Responses in VCN cells did not change to acoustic stimuli delivered correlated to licking.

In this chapter, I will discuss how the work in this thesis applies to broader questions in neuroscience. How does cancelling self-generated sounds affect mammalian auditory processing? Cerebellum-like structures are found across different species and sensory systems. How do the observations made in this thesis relate to the functions of other cerebellum-like structures? Indeed, what can we say about the cerebellum itself by studying these simpler sensory structures? How can further studies in the DCN of mice, an animal in which powerful tools for genetic and molecular manipulation of neural circuits have been developed, help answer ongoing questions of cerebellum-like structures and the cerebellum?

5.1.1. *Implications for the auditory system*

Filtering of self-generated sounds at such an early processing stage could provide mammals with a dedicated channel through which unexpected, behaviorally relevant auditory signals are used to rapidly guide behavior, such as orienting or escape. Indeed, lesioning studies of the dorsal acoustic stria, the output tract of DCN, performed in cats resulted in a deficit in the ability to orient to sounds but had no effect on the ability of cats to discriminate between sounds at two locations (Sutherland 1998; May 2000). Cancelling interfering self-generated sounds could be a key step in identifying salient auditory features of external sounds that aid in sound localization.

The acoustic startle response (ASR) is elicited by sudden acoustic stimuli (Prosser & Hunter 1936) and consists of muscle contractions in the head, neck, forelimbs, and hindlimbs with 5-10 ms latencies (Caeser et al. 1989). Such short latencies indicate that a relatively simple neuronal circuit consisting of few synapses mediates the ASR. Anatomic studies in rat have shown that the DCN projects to the pontine reticular nucleus, a brain area implicated in mediating the ASR (Lingenhöhl & Friauf 1994). Electrolytic lesioning of the DCN resulted in reduced ASRs demonstrating that the DCN is involved in mediating such a response (Meloni & M. Davis 1998). The inability to cancel self-generated sounds could cause either erroneous startle responses to predictable sounds, or a desensitization of the entire response itself.

No experiments have been performed that test the ability of DCN-lesioned animals to react to external auditory stimuli while performing behaviors that generate self-generated sounds. Such experiments could be performed in mice by performing electrolytic lesions of DCN as described by Meloni et al. (Meloni & M. Davis 1998) or lesioning the dorsal acoustic stria as performed in cats (Sutherland 1998; May 2000), or by using more modern methods such as optogenetics to selectively silence output cells of DCN. Water deprived mice could be trained to suppress licking when a soft sound is played in the midst of licking. I hypothesize that since output cells in DCN still respond to external sounds while suppressing responses to self-generated sounds, mice with intact DCNs would be able to perceive the external sound and successfully suppress their licking behavior. After DCN lesioning, mice would only have access to auditory

information conveyed by VCN. Since VCN neurons respond to both self- and externally-generated sounds, sounds due to licking would interfere with the ability to perceive a soft externally presented sound stimulus. These mice would therefore fail to successfully suppress their licking behavior in response to a soft noise.

Both the DCN and VCN project axons to the inferior colliculus (IC), specifically to the central nucleus of the inferior colliculus (Beyerl 1978; D. K. Ryugo et al. 1981; Van Noort 1969). It is not known however if both DCN and VCN project to the same cells in the IC, or to different cells maintaining a separate channel for external-sound processing. Future electrophysiological experiments in which responses to external versus self-generated sounds are characterized in IC would help answer this question.

5.1.2. *DCN and other cerebellum-like structures*

Most vertebrate brains contain sensory structures that are similar to the cerebellum in terms of their development, gene expression pattern, and circuitry. These structures include the medial octavolateral nucleus (MON) found in the lateral line system of fish (Mccormick 1999), the dorsal octavolateral nucleus (DON) found in the electrosensory system of elasmobranches, and the electrosensory lobe (ELL) found in the electrosensory system of mormyrids and gymnotids (**Figure 5.1**). Recent studies have also argued that, while not sharing a similar genetic-developmental program, the mushroom body found in the olfactory system of insects has some resemblance to other cerebellum-like structures (Farris 2011). These structures integrate a rich variety of signals conveyed via their

mossy fiber-parallel fiber system with primary sensory input. What roles do these structures play in sensory processing?

Studies performed in the ELL of mormyrids and gymnotids, and the DON of elasmobranches have provided convincing evidence that these structures act as adaptive filters to cancel out predictable, self-generated electrosensory inputs. The ELL in mormyrid fish uses proprioceptive and corollary discharge input conveyed via mossy fibers as predictive inputs to cancel electrosensory consequences of behavior (Bell 1981; Sawtell & Williams 2008; Requarth et al. 2014; Requarth & Sawtell 2014). The work done in this thesis suggests that the cerebellum-like circuitry of the DCN plays a similar role with similar adaptive filtering properties. These findings suggest that cerebellum-like sensory structures could have evolved to solve a common problem, namely the cancellation of self-generated sensory stimuli. With similar circuitry and function, one would think that these structures are homologous, evolving further from a single evolutionary event. Interestingly, this is not the case. There is no evidence for a single cerebellum-like precursor from which other cerebellum-like structures (and the cerebellum) evolved (Bell 2002).

While the lack of a single common ancestor suggests that cerebellum-like structures evolved independently through convergent evolution, such striking similarities in morphology, immunohistochemistry, and connectivity suggest that cerebellum-like structures may follow a similar genetic-developmental program. Indeed, developmental studies in which beads coated with fibroblast growth factor 8 (FGF-8) were implanted

into chick embryos showed ectopic “cerebellum-like” structures at the implantation location (Martinez et al. 1999). These ectopic structures contained cells organized in a layered manner that were similar in morphology and gene expression to Purkinje cells and granule cells. Such a genetic-developmental program could have been independently “tapped into” across phylogeny when evolutionary pressures dictated allowing for multiple, seemingly evolutionarily unrelated, cerebellum-like structures. My work provides support for the idea that function of different cerebellum-structures is also the same despite their wide phylogenetic separation.

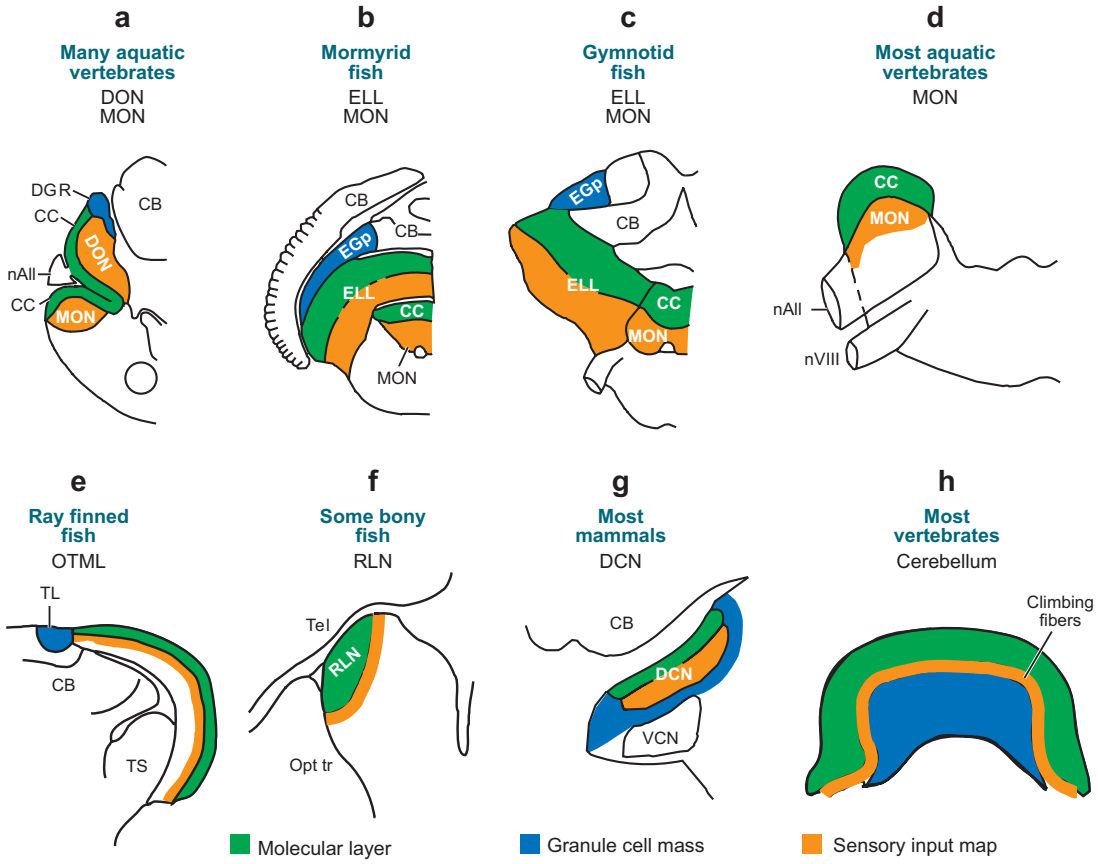

Figure 5.1| Cerebellum-like structures across vertebrate groups. Molecular layer, granule cell mass, and sensory inputs are displayed in different colors as indicated. CB, cerebellum; CC, cerebellar crest; DCN, dorsal cochlear nucleus; DGR, dorsal granular ridge; DON, dorsal octavolateral nucleus; EGp, eminentia granularis posterior; ELL, electrosensory lobe; gran, granular layer; MON, medial octavolateral nucleus; mol, molecular layer; nAll, anterior lateral line nerve; nVIII, eighth nerve; Opt tr, optic tract; RLn, rostrolateral nucleus; Tel, telencephalon; TL, torus longitudinalis; TS, torus semicircularis; VCN, ventral cochlear nucleus. Reproduced from Bell et al. 2008.

5.1.3. *Cerebellum-like structures and Cerebellum*

Can insights gained from studying cerebellum-like sensory structures be applied to the cerebellum itself? Cells in both the cerebellum and cerebellum-like structures integrate two types of inputs: parallel fiber input and a second additional input. It is the nature of the second input that differentiates the cerebellum from cerebellum-like structures. While primary sensory afferents are the additional input in cerebellum-like structures, Purkinje cells in the cerebellum integrate parallel fiber input with a single climbing fiber from the inferior olive. However, climbing fibers do respond to sensory stimuli including retinal slip (Maekawa & Simpson 1972), vestibular information (Barmack & Shojaku 1992), and somatosensory stimulation (Ekerot & Jörntell 2001). Therefore, while not primary sensory afferents, climbing fibers do convey some sensory information.

Experiments performed in Chapter 3 suggest that the DCN generates predictions of auditory consequences of behavior on the basis of proprioception. The cerebellum may also form predictions of one sensory input based on other sensory inputs. In eye-blink conditioning, a form of classical conditioning known to involve the cerebellum, the timing of an air puff to the eye is signaled by a tone. At first, eye blinks occur in response to air puffs, but after training, animals learn to time their eye blinks to occur prior to the air puff. In this case, the timing of one sensory signal, the air puff, conveyed by the climbing fiber, is predicted by another sensory signal, the tone, conveyed by mossy fibers (J. J. Kim & Thompson 1997). Mice lacking Purkinje cells or with lesions

to the deep cerebellar nuclei fail to properly time their eye blinks in response to the tone (J. J. Kim & Thompson 1997). Another example is the vestibulo-ocular reflex which stabilizes images on the retina during head movements. In this case, one sensory signal, retinal slip, is predicted by another sensory signal, vestibular information. Maekawa et al. showed climbing fiber responses in response to retinal slip (Maekawa & Simpson 1972) while evidence from Lisberger et al. (Lisberger & Fuchs 1974) suggest that vestibular signals are conveyed via mossy fibers.

Studies have shown that predictive motor control is deficient in cerebellar subjects (Morton 2006; Smith & Shadmehr 2005). For example, Morton and Bastian in 2006 (Morton 2006) performed experiments in human subjects with cerebellar damage (with healthy control subjects) in which subjects were required to walk on a split belt treadmill. Subjects began by walking on the treadmill with both belts moving at the same speed. The speed of the two belts on the treadmill were then varied such that one belt moved at a faster speed than the other. Initially, the leg on the faster belt took shorter steps, while the leg on the slower belt took longer steps. Control subjects eventually were able to adapt so that both legs took the same length steps. When the belts were changed back to moving the same speed, the leg over the slower belt took shorter steps, while the leg over the faster belt took longer steps, the opposite of the initial effect of differing belt speeds. However subjects with cerebellar damage, while able to walk normally while the belts were moving the same speed, were never able to adjust their step length when the belts moved at different speeds. They were however still able to adjust to unpredictable changes in belt speeds similar to healthy subjects. These results suggest

that the cerebellum is involved in predictive adaptations of motor control. After effects seen in healthy subjects when the belts are switched back to the same speeds are similar to negative images seen in both the ELL and DON. Despite progress towards understanding the cerebellum, many challenges remain. Sensory signals conveyed via climbing fibers are complex having already undergone much processing. Indeed some studies have argued that climbing fibers relay not all sensory input but only unexpected sensory input (Gellman et al. 1985; Devor 2000). Such inputs can be difficult to control experimentally. Cerebellum-like structures are closer to the periphery and receive less complex information directly from sensory afferents. Future studies in cerebellum-like sensory structures, including the DCN, in which inputs can be well controlled, would provide further insights into mechanisms and function of the cerebellum itself.

5.2. Future Directions

5.2.1. *Cancellation of self-generated sounds due to other behaviors*

While the results of this study focused on licking behavior, the granule cell domain of DCN receives a wide variety of mossy fiber input including the dorsal column nuclei, including the external cuneate and gracilis, (Itoh et al. 1987; Weinberg & Rustioni 1987; Saade et al. 1989), dorsal root ganglion (Zhan & Pongstaporn 2006; Kanold 2001), and the trigeminal ganglion (Shore et al. 2000; Shore 2005a). The convergence of these multiple streams of non-auditory information onto granule cells of DCN suggeststhat the DCN is likely able to cancel sensory consequences of not only licking behavior, but a

myriad of other behaviors. Previous studies in the decerebrate cat found that both simple-spiking and complex spiking cells in DCN respond to displacement of the external ear (Kanold 2001). When external sounds are played, mice often respond by a rapid movement of the external ear (Jero et al. 2009). Such movements may produce profound self-generated noise given the external ear's proximity to the tympanic membrane. Similarly, anatomic and electrophysiological studies show that the DCN receives mossy fiber input from the dorsal column nuclei, including the external cuneate nucleus, which receives somatosensory input from forelimbs (Itoh et al. 1987). Can the DCN cancel self-generated sounds due to movement of the external ear or stepping?

While experiments performed in this thesis, particularly in Chapter 3, show that the DCN uses proprioceptive input to cancel self-generated noise, studies in other cerebellum-like structures have shown that corollary discharge can also act as predictive information for sensory cancellation. Bell showed that corollary discharge signals related to the electric organ discharge conveyed to the ELL are used to cancel electrosensory consequences of that discharge (Bell 1981). Requarth et al. generalized these findings to more complex corollary discharge related to tail movement (Requarth & Sawtell 2014). Anatomic studies have shown that the DCN receives mossy fiber input from the pontine nuclei, which are known to relay motor command from the cerebral cortex to the cerebellum (Ohlrogge et al. 2001) suggesting the DCN does receive motor information. Such motor signals, in addition to proprioceptive signals conveyed by sp5, could also be involved in cancellation of self-generated sounds due to licking. Does the DCN use

corollary discharge signals as well as proprioceptive information cancel self-generated noise?

5.2.2. *Cellular sites and mechanisms of plasticity*

In Chapter 4, I showed that sensory cancellation in DCN is not static but are plastic: when an external sound is played correlated with licking, responses to that sound are decreased. These observations are similar to what has been shown previously in the electrosensory lobe (ELL) of mormyrid fish (Bell 1981). In 1993, Bell et al. (Bell et al. 1993) localized the site of plasticity to cells in the ELL by performing intracellular studies *in vivo*. In these experiments, intracellular recordings were taken from Purkinje-like cells in a paralyzed fish preparation where corollary discharge signals of the electric organ discharge (EOD command) remained intact. The authors paired depolarizing current injection at a consistent delay from the EOD command. Prior to pairing, the cells responded little to the EOD command, but after pairing, cells responded with a hyperpolarizing current to the command, an observation that was in congruence with Bell's previous study in 1981 where he paired with sensory stimulus (Bell 1981). By using current injection into single cells, the authors were able to localize the site of plasticity to fibers synapsing onto cells in ELL. Taken together, these converging lines of evidence from *in* vivo and *in vitro* experiments, and modeling studies suggest that the site of plasticity in ELL is parallel fiber synapses.

While results presented in this thesis, along with previous studies showing anti-Hebbian plasticity at parallel fiber synapses (Tzounopoulos et al. 2004; Zhao & Tzounopoulos 2011) suggest that DCN cells are the sites of plasticity, this has not been confirmed. A conceptually simple, though technically difficult, experiment would be to perform intracellular recordings in simple spiking cells from awake, licking mice. Similar to experiments described above, rather than pair external noise to licking, current injections into single cells would be paired. If fusiform cells in DCN were the sites of plasticity, a depression in membrane potential would be seen in response to licking. The timing of the depression would be the same as the timing of the paired current injection.

This experiment may also be able to reveal negative images. Since parallel fibers also carry auditory information, pairing with sound not only activates auditory nerve fibers, but also a different pattern of parallel fiber inputs. This would result in different parallel fibers being active when the mouse is licking without sound than with the paired sound. Therefore, observing negative images is potentially problematic by simply removing the paired sound. By pairing with intracellular current injections, the pattern of activation of parallel fibers while the mouse is licking would be the same before, during, and after pairing allowing for negative images to be observed by simply removing the paired current injection. Such a result would show a common mechanism of sensory cancellation across cerebellum-like structures, namely the formation of negative images.

Recordings in brain slices showed that anti-Hebbian plasticity in DCN at both parallel fiber-cartwheel cell and parallel fiber-fusiform cell synapses is endocannabinoid

mediated (Tzounopoulos et al. 2007; Zhao & Tzounopoulos 2011). Similar mechanisms may also be involved in sensory cancellation observed *in vivo.* To test this hypothesis, similar experiments performed in Chapter 4 could be repeated in a mouse injected with AM251, an endocannabinoid receptor antagonist. When AM251 was applied in slice preparations, pairing protocols that would normally cause LTD caused LTP (Tzounopoulos et al. 2007; Zhao & Tzounopoulos 2011). *In vivo*, increases in responses to noise played correlated to licking may be observed rather than decreases or no change. These experiments would link observations from *in vitro* studies with those made in Chapter 4 of this thesis.

Mouse brain slice studies also showed two different plasticity rules at parallel fiber-fusiform cell synapses. As described in Chapter 4, early experiments originally showed a Hebbian learning rule (Tzounopoulos et al. 2004) however, after stimulation of cholinergic input (the olivocochlear bundle), parallel fiber-fusiform cell synapses showed an anti-Hebbian learning rule. One hypothesized function of the olivocochlear bundle is the "unmasking" of transient auditory stimuli in the presence of constant background noise by reducing auditory nerve responses to background noise. (P. C. Nieder & I. Nieder 1970; Kawase et al. 1993). The olivocochlear bundle could be playing a similar role in the DCN, namely reducing responses to self-generated sounds. Future experiments could test if olivocochlear inputs are necessary to produce the suppression effects seen in Chapter 4. By expressing channel rhodopsin or halorhodopsin in the superior olivary complex SOC, the source of the olivocochlear bundle), one would be able to transiently excite or suppress the bundle prior to pairing.

Further experiments may also provide insights into the function of Hebbian learning at parallel fiber-fusiform cell synapses. Experiments done in both humans and rats have shown that sound can enhance perception of touch (Ro et al. 2009; Lak et al. 2008). Since self-generated sounds of active touching (whisking against textured surfaces) may aid in the ability to discriminate the surface, there may be times when animals want to specifically hear self-generated sounds. In this case, Hebbian learning at parallel fiber-fusiform cell synapses would enhance responses to self-generated sounds. What are the natural conditions for Hebbian versus anti-Hebbian learning at these synaspes?

5.2.3. *What role do Purkinje-like cells play?*

Since Purkinje-like cells (the medium ganglion or MG cell in ELL and cartwheel cells in DCN) form inhibitory synapses with efferent cells and form a large number of synapses with parallel fibers, one may expect that they play an important role in cancellation of self-generated sensory input. However, in ELL, efferent cells form negative images when corollary discharge inputs are paired with depolarizing intracellular current injections (Bell, Caputi, et al. 1997) showing that MG cells are not necessary for the change in responses seen in efferent cells of ELL, at least under the conditions of these experiments. Further, since ELL efferent cells receive primary sensory input and parallel fiber input (Grant et al. 1996), and show evidence of plasticity at their parallel fiber synapses (Bell, Caputi, et al. 1997), they already have the

ingredients necessary to cancel sensory consequences of behavior. Similarly, fusiform cells, efferent cells of the DCN, also receive primary sensory input and parallel fiber input, and have similar anti-Hebbian plasticity at their parallel fiber synapses. Since Purkinje-like cells may not be necessary in the generation of negative images, or possibly sensory cancellation, in efferent cells, what role do they play in the function of cerebellum-like structures?

To help answer this question, the myriad of molecular and genetic techniques developed for the mouse is particularly advantageous. A mouse line with a spontaneous mutation that lacks Purkinje cells called *Purkinje cell degeneration (PCD)* (Mullen et al. 1976). Since cartwheel cells and Purkinje cells share many similarities, including similarities in gene expression, cartwheel cells also degenerate in this line (Berrebi & Morgan 1990). Units recorded in the DCN of anesthetized *pcd* mice showed no difference in response to tones and noise, spontaneous rates or thresholds as compared to controls (Parham et al. 2000) showing that cartwheel cells may not play a role in shaping responses of output cells to sound alone. Since cartwheel cells, and indeed all Purkinje-like cells receive parallel fiber input which convey a variety of information, further studies in awake, behaving *pcd* mice, in which the wide array of parallel fibers would be naturally engaged, would provide more information on the role of cartwheel cells.

Another option would be to transiently silence – or activate – cartwheel cells using optogenetic techniques. Multiple genetic markers label only cartwheel cells in DCN (and Purkinje cells in the cerebellum) including cerebellin (Mugnaini & Morgan

1987) and PEP-19 (also known as Purkinje Cell Protein 4, or PCP4) (Mugnaini et al. 1987). This would allow selective activation or silencing of cartwheel cells. For example either channelrhodopsin or halorhodopsin under the influence of a PEP-19 promoter could be expressed only in cartwheel cells of DCN. These experiments would help elucidate the roles of cartwheel cells, and provide insights to the role of Purkinje-like cells in other cerebellum-like structures, in sensory processing.

5.3. Concluding Remarks

This thesis found that the dorsal cochlear nucleus cancels sensory consequences to the animal's own behavior. Chapter 2 showed that the DCN suppresses responses to self-generated noise due to licking behavior while remaining sensitive to external auditory stimuli. Chapter 3 showed that licking behavior engages the mossy fiber-parallel fiber system and that mossy fiber inputs from the spinal trigeminal nucleus are integral to the cancellation seen in Chapter 2. Finally, Chapter 4 showed sensory cancellation in DCN is not static but can change. These results demonstrate that the DCN shares similar functions to other cerebellum-like sensory structures and suggests that while evolutionarily distinct, cerebellum-like structures may play similar roles across phylogeny. Studying cerebellum-like structures in mouse unlocks the vast array of genetic and molecular techniques that have been developed to probe neural circuits. Further studies in mouse DCN will provide important insights into mechanisms and function of neural circuits of other cerebellum-like structures and indeed, the cerebellum itself.

Chapter 6 - Methods

Animals

Adult male wild-type mice (129S6/SvEvTac) were used for all experiments. Mice were purchased from Taconic Biosciences (Hudson, NY) and housed in an on-site animal facility.

Surgery

Mice were anesthetized with isoflurane (1.5-2%) and placed in a stereotax equipped with zygomatic ear bars (Kopf Instruments). The skull was exposed and a small craniotomy 200-500 μm in diameter was made over the right dorsal cochlear nucleus (5.5 mm posterior to bregma and 2.3mm lateral to the midline). The craniotomy was covered with silicon elastomer (Kwik-Sil, WPI, Sarasota, FL). A custom headplate was attached to the skull using dental cement (C&B Meta-bond, Parkell, Edgewood, NY). Mice were allowed to recover for 3 days prior to the start of experiments.

Experimental apparatus and auditory stimulus presentation

All mouse behavior and neurophysiology experiments were performed in a double walled sound-attenuating chamber (Double Deluxe Model, Gretchken Industries). The ambient noise within the chamber was <30 dB SPL as measured by a sound pressure level meter (Bruel and Kjaer Type 2240). A custom head fixation device was used to secure the animal via two attachment points to a stainless steel headplate and allowed for consistent

positioning across multiple recording sessions. The animal's body was additionally secured between two pieces of styrofoam molded to its body. A stainless steel lick spout was positioned in front of the animal's mouth and licks were detected by simply connecting a metal lick spout to the input of the analog-digital converter (Power 1401, Cambridge Electronic Design) as first described by Hayar et al (Hayar et al. 2006). Tongue-to-spout contacts caused large, easily identifiable DC voltage steps. Acoustic stimuli were generated using Spike2 software (Cambridge Electronic Design) and delivered using an electrostatic speaker (ES-1Tucker Davis Technologies) positioned approximately 10 cm in front of the mouse just to the right of the midline. Sound pressure levels of acoustic stimuli as measured in dB SPL were calibrated to the location of the animal's right ear. The frequency response of the sound system was measured to be flat (+/- 4dB) from 1kHz to 50kHz using a ¼'' condenser microphone (377C01, PCP piezotronics), attached to a preamplifier (426B03, PCP piezotronics) positioned at the location of the mouse's right ear. Sounds caused by licking were monitored by small electret microphone (Knowles model 23329N) placed just above the lick spout. Microphone signals were sampled at 100 kHz and digitized using an analog to digital converter (Power 1401, Cambridge Electronic Design).

The mimic of the licking sound was constructed from segments of microphone recordings 50 ms before tongue contact to 150 ms after tongue contact, and bandpass filtered between 1 and 50 kHz. We transformed each segment to a spectrogram using a short-time Fourier transform (Hamming window with a width of 10.24 ms and a stride of 5.12 ms). We then constructed the mimic by performing PCA on this set of lick-triggered

spectrograms and making a weighted sum of the first five principal components. This resulted in a mimic spectrogram, which we used as a spectro-temporal filter to convolve with a random signal. This resulted in a stimulus (mimic) which contained the most prominent spectro-temporal features of the licking sound (including distinct spectral peaks at 2, 8, and 30 kHz) with little power elsewhere.

Behavioral training

Mice were allowed to recover 3 days after surgery before beginning water deprivation and habituation to head restraint in the experimental apparatus. Weight was monitored daily and additional water was given in the home cage if the animals' weight fell below 80% of its initial pre-surgical weight. Extracellular recordings from DCN and VCN units were then performed during daily sessions lasting 2-3 hours. Mice licked roughly 3,000 times per session.

Extracellular recording and identification of DCN and VCN neurons

Standard procedures were used for extracellular recording using glass microelectrodes filled with 2M NaCl (5-20 MΩ resistance). Pipettes with a long taper were used to avoid tissue damage. On the day of recording, mice were placed into the head restraint and the silicone elastomer was removed and 0.9% saline was placed over the exposed craniotomy. The microelectrode was lowered into the craniotomy vertically. As the electrode was advanced through the cerebellum a series of 200 ms long search tones from 5 kHz to 50 kHz (in 5 kHz steps) were delivered. Entrance into DCN was marked by a transient increase in electrode resistance along with the sudden appearance of tone-evoked multi-

unit activity which occurred ~2700-3200 μm below the surface of the cerebellum. The microelectrode was then advanced in 1 um steps until a unit was isolated. Water was given only after a unit was isolated. Complex-spiking units were the first units encountered on an electrode penetration through DCN and could be unambiguously identified based on their distinctive complex spikes. Complex spikes are stereotyped, high-frequency action potential bursts superimposed on a slower depolarization and are not observed in any DCN cell types except CWCs (Manis et al. 1994; Zhang & Oertel 1993). Similar to previous *in vivo* extracellular recording studies of DCN in a variety of species, including mouse (Ma & Brenowitz 2012), we defined complex spikes as high-frequency bursts (ISIs < 3.5 ms) of 2-5 action potentials. Complex spikes were identified automatically in Spike2 using custom written scripts and then confirmed individually. Within such bursts, action potentials successively widened and decreased in amplitude (**Fig. 2b**). Complex-spiking units were isolated 50-200 μm from the surface of the DCN. DCN units lacking complex spikes, referred to here as simple spiking units, were isolated 100-300 μm from the surface of the DCN. Complex-spiking units were never found ventral to simple spiking units on the same electrode penetration consistent with the known cytoarchitecture of the DCN. Passage from DCN into VCN was determined by monitoring the tone frequency that most strongly drove multi-unit activity for each 50 μm advance of the electrode. As the electrode advanced ventrally, the best frequency for driving multi-unit activity progressively decreased. A sudden increase in the best frequency (generally from ~5k Hz to ~20 kHz and usually occurring 500-600 um below the surface of DCN) signified entrance into the VCN. Units which were isolated at least 100 um ventral to the best frequency reversal (~800-1000 um below the surface of the

DCN) and which showed clear tone-evoked responses were classified as VCN units. Units isolated less than 100 um of the best frequency reversal were not included in the analysis. Histological verification of DCN and VCN recording sites was performed by iontophoresis of dextran conjugated Alexa Flour 594 (D22913, Thermo Fisher Scientific) at recording sites between 100 and 300 μm below the surface of DCN (depths at which most DCN simple spiking units were isolated) and at 900 um (the depth at which most VCN units were isolated). Only units that remained well-isolated through at least 75 licks were included in the analysis. Sounds associated with licking contain most power between 2-15 kHz, which corresponds to the lower portion of the mouse hearing range. For this reason we focused our recordings on regions of the cochlear nucleus that represent these frequencies.

Viral Injections

A nanoliter injector (504126, WPI instruments) was used to inject adeno-associated virus expressing green fluorescent protein. The pipette was positioned over the coordinates 7.2 mm posterior to bregma and 1.8 mm right of the midline and lowered until the tip touched the surface of the cerebellum. The pipette was then lowered 3.5 mm below the surface of the cerebellum to the base of the spinal trigeminal nucleus. 27 μL of the virus was injected in three 9 μL pulses. Virus was also injected at depths of 3.2, 2.9, and 2.7 mm below the surface of the cerebellum. The pipette was then slowly raised out of the cerebellum and the incision was closed using cyanoacrylate glue (Vetbond, 3M, Maplewood Minnesota). Two weeks after surgery, mice were anesthetized with ketamine/xylazine and perfused with 4% formaldehyde. The brains were dissected from

the skull and allowed to post-fix in 4% formaldehyde overnight. They were then cryoprotected in a 30% sucrose solution and sectioned on a cryostat. Sections were then mounted on glass slides (Superfrost, Fisher Scientific, Waltham, MA), counterstained with DAPI, and imaged on a confocal microscope (Carl Zeiss Microscopy, Peabody, MA).

Deafening

Mice were deafened bilaterally. Surgery for deafening mice was performed 2-4% isoflurane. An incision made just posterior to the tragus and extended ventrally. The tympanum, malleus, and incus were visualized through the auditory meatus. Using fine forceps the tympanum was ruptured and the malleus and incus were removed. The stapes was removed exposing the oval window with care taken not to damage the stapedial artery. Using a 30 gauge needle, approximately 10-20 μL of 1.0 mg/mL kanamycin was injected through the oval window and into the cochlea. The middle ear was packed with gel foam and the mouse was allowed to recover in its home cage. Deafening was verified by recording sound evoked field potentials to broadband noise (50 ms, 6-90 dB SPL) in DCN ~75 μm below the first observed complex-spiking unit. This was done both before and 2 days after surgical deafening in each mouse. DCN recordings were performed 2-4 days after surgery. Recording locations within DCN were confirmed histologically using iontophoresis of dextran-conjugated Alexa 594 as described above.

Inactivating spinal trigeminal nucleus

A small craniotomy (~300 μm diameter) was made prior to attachment of the headplate at coordinates 7.2 mm posterior to bregma and 1.8 mm lateral to the midline and covered with silicon elastomer. On the day of the experiment, a glass micropipette with a long taper was pulled using a pipette puller (PC-10, Narishige Group) and manually broken to 3.5 um diameter under a microscope. The pipette was then filled with 2% lidocaine in 0.9% saline with care taken to avoid air bubbles in the tip. The pipette was then coupled to a micropressure injector (Pikospritzer MK III, Parker Instrumentation) and successful ejection of lidocaine was confirmed visually to ensure tip was not clogged. The lidocaine pipette was advanced into Sp5 at an angle of 12.8 degrees. For Sp5 inactivation DCN unit responses were recorded for ~200 licks before ~100 nL of lidocaine was injected in a single pulse. Location of the lidocaine pipette within DCN was verified histologically using iontophoresis of dextran-conjugated Alexa 594 as described above.

Lick-sound pairing

After isolation of a unit, access to water was given and contact to the lick spout by the animal's tongue was paired with a 30 ms noise (15 dB SPL, bandpassed filtered 5-15 kHz). In the correlated condition the noise was presented 30 ms after contact with the lick spout. The pairing was conducted continuously until the animal stopped licking or unit isolation was lost. In the uncorrelated condition presentation of the noise during licking was unrelated to the tongue's contact with the spout and was instead presented at random intervals of 120-160 ms. Since these intervals are similar to inter-lick intervals the overall rate of sound presentations was similar in the correlated and uncorrelated conditions.

Data analysis

All analyses were performed using custom written scripts for Matlab (Mathworks, Natick, MA) and Spike 2.

Lick sound spectrograms: To compute the average spectrogram of the sound associated with licking we first bandpass filtered raw microphone traces removing frequencies below 1 kHz and above 50 kHz (the highest frequency that could be detected by our equipment). 300 ms segments of the filtered microphone recording centered on the onset of each lick were transformed with a short time Fourier transform (Hamming window with a width of 10.24 ms and a stride of 5.12 ms) to obtain a set of lick-centered spectrograms. These were averaged to obtain a lick-triggered average spectrogram. Time-frequency peaks were found by first applying a 2-D median filter (widths 290 Hz, 3 ms) to individual spectrograms and then convolving with a 2-D Gaussian kernel with widths 1.5 kHz and 20 ms. We then calculated local time-frequency maximums by finding local maximums of the filtered spectrograms.

RMS of microphone traces: To compute the RMS of the sound associated with licking microphone recordings were first bandpass filtered (1-50 kHz). We then computed the RMS of this filtered microphone trace by convolving the squared trace with a moving average kernel of width 1 ms and taking the square root of the result. These recordings were then aligned to the tie of tongue-to-spout contact and averaged across licks.

Average and Z-scored electrophysiological responses during licking and mimic presentation: To compute average responses to licking or during delivery of the mimic spike trains were convolved with a normalized sum-of-two-exponentials kernel, with a rise time of 5 ms and a decay time of 20 ms. Averages were aligned either on tongue contact with the lick spout or mimic delivery and average baseline firing was subtracted. Baseline firing rates was taken to be the average firing rates in periods at least 25 ms before the next lick or mimic onset and at least 150 ms after the previous lick or mimic onset. Peak-to-trough firing rates were computed by taking the average licking or mimic response in a 200 ms window centered on the tongue-to-spout contact or mimic onset and determining the difference in the maximum to minimum firing rates. To compute z-scores we first took the maximum of the average licking or mimic response in a 200 ms window centered on tongue-to-spout contract or mimic onset. We then created shuffled spike trains of approximately the same length as the original spike train by randomly sampling from the inter-spike-interval distribution of the real spike train. Each shuffled spike train was convolved with the same kernel as the real spike train, its lick- or mimic-triggered average computed, and the maximum firing rate of this triggered average taken in the same 200 ms window. This was repeated 500 times and the maximum of the triggered average of the real spike train was expressed in units of the standard deviation from the mean of the shuffle distribution, i.e. z-scored based on the shuffle distribution. Z-scores greater than or equal to 2.33 were considered significant.

Correlated and uncorrelated sound-lick pairings: Noise presentations were binned into groups of 150. In each bin we computed the average firing rate in a window from -50ms

to 80ms around the onset of the noise and determined the peak to trough of the firing rate. We then normalized the peak to trough firing rate in each bin to the peak to trough firing rate in the first 150 noise presentations of the experiment (called the normalized noise response). We then performed linear regression analysis to test the correlation between noise presentations and noise response in the correlated and uncorrelated conditions.

Bibliography

Adams, J.C., 1979. Ascending projections to the inferior colliculus. *Journal of Comparative Neurology*.

Anderson, L.A. et al., 2009. A monosynaptic pathway from dorsal cochlear nucleus to auditory cortex in rat. *Neuroreport*, 20(5), pp.462–466.

Apostolides, P.F. & Trussell, L.O., 2014. Superficial stellate cells of the dorsal cochlear nucleus. *Frontiers in neural circuits*, 8, p.63.

Balakrishnan, V. & Trussell, L.O., 2008. Synaptic inputs to granule cells of the dorsal cochlear nucleus. *Journal of Neurophysiology*, 99(1), pp.208–219.

Barmack, N.H. & Shojaku, H., 1992. Representation of a Postural Coordinate System in the Nodulus of the Rabbit Cerebellum by Vestibular Climbing Fiber Signals. In *Vestibular and Brain Stem Control of Eye*. S. Karger AG, pp. 331–338.

Bastian, J., 1995. Pyramidal-cell plasticity in weakly electric fish: a mechanism for attenuating responses to reafferent electrosensory inputs. *Journal of Comparative Physiology A: Neuroethology*.

Bell, C. et al., 2004. The Generation and Subtraction of Sensory Expectations within Cerebellum-Like Structures. *Brain, Behavior and Evolution*, 50(1), pp.17–31.

Bell, C.C., 1981. An efference copy which is modified by reafferent input. *Science*, 214(4519), pp.450–453. Available at: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=7291985&retmode=ref&cmd=prlinks.

Bell, C.C., 2002. Evolution of Cerebellum-Like Structures. *Brain, Behavior and Evolution*, 59(5-6), pp.312–326.

Bell, C.C. & Russell, C.J., 1978. Effect of electric organ discharge on ampullary receptors in a mormyrid. *Brain Research*.

Bell, C.C. et al., 1993. Storage of a sensory pattern by anti-Hebbian synaptic plasticity in an electric fish. *Proceedings of the National Academy of Sciences of the United States of America*, 90(10), pp.4650–4654.

Bell, C.C., Caputi, A. & Grant, K., 1997. Physiology and Plasticity of Morphologically Identified Cells in the Mormyrid Electrosensory Lobe. *The Journal of Neuroscience*.

Bell, C.C., Grant, K. & Serrier, J., 1992. Sensory processing and corollary discharge effects in the mormyromast regions of the mormyrid electrosensory lobe. I. Field potentials, cellular

activity in associated structures. *Journal of Neurophysiology*, 68(3), pp.843–858.

Bell, C.C., Han, V. & Sawtell, N.B., 2008. Cerebellum-Like Structures and Their Implications for Cerebellar Function. *Annual Review of Neuroscience*, 31(1), pp.1–24.

Bell, C.C., Han, V.Z., et al., 1997. Synaptic plasticity in a cerebellum-like structure depends on temporal order. *Nature*, 387(6630), pp.278–281.

Berrebi, A. & Morgan, J., 1990. The Purkinje cell class may extend beyond the cerebellum. *Journal of neurocytology*.

Berrebi, A.S. & Mugnaini, E., 1991. Distribution and targets of the cartwheel cell axon in the dorsal cochlear nucleus of the guinea pig. *Anatomy and embryology*, 183(5), pp.427–454.

Beyerl, B.D., 1978. Afferent projections to the central nucleus of the inferior colliculus in the rat. *Brain Research*.

Blackstad, T.W., Osen, K.K. & Mugnaini, E., 1984. Pyramidal neurones of the dorsal cochlear nucleus: a Golgi and computer reconstruction study in cat. *NSC*, 13(3), pp.827–854.

Bodznick, D., 1993. The Specificity of an Adaptive Filter That Suppresses Unwanted Reafference in Electrosensory Neurons of the Skate Medulla. *The Biological Bulletin*, 185(2), pp.312b–314.

Bodznick, D. & MONTGOMERY, J.C., 1992. Suppression of Ventilatory Reafference in the Elasmobranch Electrosensory System: Medullary Neuron Receptive Fields Support a Common Mode Ejection Mechanism. *Journal of experimental biology*, 171(1), pp.127–137.

Bodznick, D., MONTGOMERY, J.C. & BRADLEY, D.J., 1992. Suppression of Common Mode Signals Within the Electrosensory System of the Little Skate Raja Erinacea. *Journal of experimental biology*, 171(1), pp.107–125.

Borges-Merjane, C. & Trussell, L.O., 2015. ON and OFF unipolar brush cells transform multisensory inputs to the auditory system. *Neuron*, 85(5), pp.1029–1042.

Brawer, J.R., Morest, D.K. & Kane, E.C., 1974. The neuronal architecture of the cochlear nucleus of the cat. *The Journal of Comparative Neurology*, 155(3), pp.251–299.

Brown, M.C. et al., 1988. Central trajectories of type II spiral ganglion neurons. *The Journal of Comparative Neurology*, 278(4), pp.581–590.

Burian, M., Gstoettner, W. & Zundritsch, R., 1989. Saccular afferent fibers to the cochlear nucleus in the guinea pig. *Archives of oto-rhino-laryngology*, 246(5), pp.238–241.

Caeser, M., Ostwald, J. & Pilz, P.K., 1989. Startle responses measured in muscles innervated by facial and trigeminal nerves show common modulation. *Behavioral Neuroscience*, 103(5), p.1075.

Cajal, S.Y., 1911. *Histogénèse du cervelet*, Histologie du Système Nerveux de l ….

Cant, N.B. & Casseday, J.H., 1986. Projections from the anteroventral cochlear nucleus to the lateral and medial superior olivary nuclei. *The Journal of Comparative Neurology*, 247(4),

pp.457–476.

Crapse, T.B. & Sommer, M.A., 2008. Corollary discharge across the animal kingdom. *Nature Reviews Neuroscience*, 9(8), pp.587–600.

Davis, K. & Miller, R., 1996. Effects of somatosensory and parallel-fiber stimulation on neurons in dorsal cochlear nucleus. *Journal of Neurophysiology*.

Davis, K.A. & Young, E.D., 1997. Granule cell activation of complex-spiking neurons in dorsal cochlear nucleus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 17(17), pp.6798–6806.

Davis, K.A. et al., 1996. Response properties of units in the dorsal cochlear nucleus of unanesthetized decerebrate gerbil. *Journal of Neurophysiology*, 75(4), pp.1411–1431.

De No, R.L., 1981. *The primary acoustic nuclei*,

de Nó, R.L., 1933. Anatomy of the eighth nerve: III.—General plan of structure of the primary cochlear nuclei. *The Laryngoscope*.

de Nó, R.L., 1979. *Central representation of the eighth nerve* V. Goodhill, ed., Hagerstown, Maryland: Ear diseases, deafness, and dizziness.

Devor, A., 2000. Is the cerebellum like cerebellar-like structures? *Brain Research Reviews*.

Ekerot, C.F. & Jörntell, H., 2001. Parallel fibre receptive fields of Purkinje cells and interneurons are climbing fibre-specific - Ekerot - 2001 - European Journal of Neuroscience - Wiley Online Library. *European Journal of Neuroscience*.

Eliades, S.J. & Wang, X., 2002. Sensory-Motor Interaction in the Primate Auditory Cortex During Self-Initiated Vocalizations. *Journal of Neurophysiology*, 89(4), pp.2194–2207.

Engelmann, J. et al., 2008. Electric imaging through active electrolocation: implication for the analysis of complex scenes. *Biological Cybernetics*, 98(6), pp.519–539.

Farago, A.F., Awatramani, R.B. & Dymecki, S.M., 2006. Assembly of the Brainstem Cochlear Nuclear Complex Is Revealed by Intersectional and Subtractive Genetic Fate Maps. *Neuron*, 50(2), pp.205–218.

Farris, S.M., 2011. Are mushroom bodies cerebellum-like structures? *Arthropod structure & development*, 40(4), pp.368–379.

Fernandez, C. & Karapas, F., 1967. The course and termination of the striae of Monakow and held in the cat. *The Journal of Comparative Neurology*, 131(3), pp.371–385.

Fernandez-Gonzalez, A. & La Spada, A.R., 2002. Purkinje cell degeneration (pcd) phenotypes caused by mutations in the axotomy-induced gene, Nna1. ....

Floris, A. et al., 1994. The unipolar brush cells of the rat cerebellar cortex and cochlear nucleus are calretinin-positive: a study by light and electron microscopic immunocytochemistry. *Anatomy and embryology*, 189(6).

Fujino, K. & Oertel, D., 2002. Bidirectional synaptic plasticity in the cerebellum-like mammalian dorsal cochlear nucleus. *Proceedings of the National Academy of Sciences*, 100(1), pp.265–270.

Fünfschilling, U. & Reichardt, L.F., 2002. Cre-mediated recombination in rhombic lip derivatives. *Genesis (New York, N.Y. : 2000)*, 33(4), pp.160–169.

Gardner, M.B., 1973. Problem of localization in the median plane: effect of pinnae cavity occlusion. *The Journal of the Acoustical Society of America*, 53(2), pp.400–408.

Gellman, R., Gibson, A.R. & Houk, J.C., 1985. Inferior olivary neurons in the awake cat: detection of contact and passive body displacement. *Journal of Neurophysiology*, 54(1), pp.40–60.

Golding, N.L. & Oertel, D., 1997. Physiological identification of the targets of cartwheel cells in the dorsal cochlear nucleus. *Journal of Neurophysiology*, 78(1), pp.248–260.

Grant, K. et al., 1996. Projection neurons of the mormyrid electrosensory lateral line lobe: morphology, immunohistochemistry, and synaptology. *Journal of ...*.

Haenggeli, C.A. & Pongstaporn, T., 2005. Projections from the spinal trigeminal nucleus to the cochlear nucleus in the rat - Haenggeli - 2005 - Journal of Comparative Neurology - Wiley Online Library. *Journal of ...*.

Hamilton, B.A. et al., 1996. Disruption of the nuclear hormone receptor RORα in staggerer mice. *Nature*.

Hancock, K.E. & Voigt, H.F., 2002. Intracellularly labeled fusiform cells in dorsal cochlear nucleus of the gerbil. II. Comparison of physiology and anatomy. *Journal of Neurophysiology*, 87(5), pp.2520–2530.

Hayar, A. et al., 2006. A low-cost solution to measure mouse licking in an electrophysiological setup with a standard analog-to-digital converter. *Journal of Neuroscience Methods*, 153(2), pp.203–207.

Hirsch, J.A. & Oertel, D., 1988. Synaptic connections in the dorsal cochlear nucleus of mice, in vitro. *The Journal of physiology*, 396, pp.549–562.

Holst, von, E. & Mittelstaedt, H., 1950. The Reafference Principle. *Die Naturwissenschaften*, 37(20), pp.464–476.

Itoh, K. et al., 1987. Direct projections from the dorsal column nuclei and the spinal trigeminal nuclei to the cochlear nuclei in the cat. *Brain Research*, 400(1), pp.145–150.

Jero, J., Coling, D. & Lalwani, A.K., 2009. The Use of Preyer's Reflex in Evaluation of Hearing in Mice. *Acta Oto-Laryngologica*, 121(5), pp.585–589.

Kane, E.C., 1974. Synaptic organization in the dorsal cochlear nucleus of the cat: a light and electron microscopic study. *Journal of Comparative Neurology*.

Kanold, P., 2001. Proprioceptive information from the pinna provides somatosensory input to cat

dorsal cochlear nucleus. *The Journal of Neuroscience*.

Kanold, P.O., Davis, K.A. & Young, E.D., 2011. Somatosensory context alters auditory responses in the cochlear nucleus. *Journal of Neurophysiology*, 105(3), pp.1063–1070.

Kawase, T., Delgutte, B. & Liberman, M.C., 1993. Antimasking effects of the olivocochlear reflex. II. Enhancement of auditory-nerve response to masked tones. *Journal of Neurophysiology*, 70(6), pp.2533–2549.

Kim, A.J., Fitzgerald, J.K. & Maimon, G., 2015. Cellular evidence for efference copy in Drosophila visuomotor processing. *Nature Publishing Group*, 18(9), pp.1247–1255.

Kim, J.J. & Thompson, R.E., 1997. Cerebellar circuits and synaptic mechanisms involved in classical eyeblink conditioning. *Trends in Neurosciences*.

Koehler, S.D. & Shore, S.E., 2013. Stimulus-Timing Dependent Multisensory Plasticity in the Guinea Pig Dorsal Cochlear Nucleus. *PLOS ONE*, 8(3), p.e59828.

Koehler, S.D. et al., 2011. Somatosensory inputs modify auditory spike timing in dorsal cochlear nucleus principal cells. *The European journal of neuroscience*, 33(3), pp.409–420.

Lak, A., Arabzadeh, E. & Diamond, M.E., 2008. Enhanced Response of Neurons in Rat Somatosensory Cortex to Stimuli Containing Temporal Noise. *Cerebral Cortex*.

Lauer, A.M., Slee, S.J. & May, B.J., 2011. Acoustic Basis of Directional Acuity in Laboratory Mice. *Journal of the Association for Research in Otolaryngology*, 12(5), pp.633–645.

Laurie, D.J., Seeburg, P.H. & Wisden, W., 1992. The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. II. Olfactory bulb and cerebellum. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 12(3), pp.1063–1076.

Lingenhöhl, K. & Friauf, E., 1994. Giant neurons in the rat reticular formation: a sensorimotor interface in the elementary acoustic startle circuit? *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 14(3 Pt 1), pp.1176–1194.

Lisberger, S.G. & Fuchs, A.F., 1974. *Lisberger: Response of flocculus Purkinje cells to... - Google Scholar*, Brain research.

Luo, F. et al., 2009. Three-dimensional tonotopic organization of the C57 mouse cochlear nucleus. *Hearing research*, 257(1-2), pp.75–82.

Ma, W.-L.D. & Brenowitz, S.D., 2012. Single-neuron recordings from unanesthetized mouse dorsal cochlear nucleus. *Journal of Neurophysiology*, 107(3), pp.824–835.

Maekawa, K. & Simpson, J.I., 1972. Climbing fiber activation of Purkinje cells in the flocculus by impulses transferred through the visual pathway. *Brain Research*, 39(1), pp.245–251.

Malmierca, M.S. et al., 2002. Direct projections from cochlear nuclear complex to auditory thalamus in the rat. *Journal of Neuroscience*, 22(24), pp.10891–10897.

Manis, P.B. et al., 1994. Physiology and morphology of complex spiking neurons in the guinea pig dorsal cochlear nucleus - Manis - 2004 - Journal of Comparative Neurology - Wiley

Online Library. *The Journal of Comparative Neurology*, 348(2), pp.261–276.

Martinez, S. et al., 1999. FGF8 induces formation of an ectopic isthmic organizer and isthmocerebellar development via a repressive effect on Otx2 expression. *Development*, 126(6), pp.1189–1200.

May, B.J., 2000. Role of the dorsal cochlear nucleus in the sound localization behavior of cats. *Hearing research*.

Mccormick, C.A., 1999. Anatomy of the Central Auditory Pathways of Fish and Amphibians. In *Comparative hearing: Fish and amphibians*. Springer Handbook of Auditory Research. New York, NY: Springer New York, pp. 155–217.

Meloni, E.G. & Davis, M., 1998. The dorsal cochlear nucleus contributes to a high intensity component of the acoustic startle reflex in rats. *Hearing research*.

MONTGOMERY, J.C., 1984. Noise cancellation in the electrosensory system of the thornback ray; common mode rejection of input produced by the animal's own ventilatory movement. *Journal of Comparative Physiology A*, 155(1), pp.103–111.

Morton, S.M., 2006. Cerebellar Contributions to Locomotor Adaptations during Splitbelt Treadmill Walking. *Journal of Neuroscience*, 26(36), pp.9107–9116.

Mugnaini, E., 1985. GABA neurons in the superficial layers of the rat dorsal cochlear nucleus: Light and electron microscopic immunocytochemistry. *The Journal of Comparative Neurology*, 235(1), pp.61–81.

Mugnaini, E. & Morgan, J.I., 1987. The neuropeptide cerebellin is a marker for two similar neuronal circuits in rat brain. *Proceedings of the National …*.

Mugnaini, E. & Warr, W., 1980. Distribution and light microscopic features of granule cells in the cochlear nuclei of cat, rat, and mouse. *The Journal of Comparative …*.

Mugnaini, E. et al., 1987. The polypeptide PEP-19 is a marker for Purkinje neurons in cerebellar cortex and cartwheel neurons in the dorsal cochlear nucleus. *Archives italiennes de biologie*, 126(1), pp.41–67.

Mugnaini, E., Osen, K. & Dahl, A., 1980. Fine structure of granule cells and related interneurons (termed Golgi cells) in the cochlear nuclear complex of cat, rat and mouse. *Journal of neurocytology*.

Mullen, R.J., Eicher, E.M. & Sidman, R.L., 1976. Purkinje cell degeneration, a new neurological mutation in the mouse.

Muniak, M.A. et al., 2013. 3D model of frequency representation in the cochlear nucleus of the CBA/J mouse. *The Journal of Comparative Neurology*, 521(7), pp.1510–1532.

Musicant, A.D., 1990. Direction-dependent spectral properties of cat external ear: New data and cross-species comparisons. *The Journal of the Acoustical Society of America*, 87(2), pp.757–781.

Nazruddin et al., 1989. The cells of origin of the hypoglossal afferent nerves and central projections in the cat. *Brain Research*, 490(2), pp.219–235.

Nelson, A. et al., 2013. A circuit for motor cortical modulation of auditory cortical activity. *Journal of Neuroscience*, 33(36), pp.14342–14353.

Nieder, P.C. & Nieder, I., 1970. *Nieder: Crossed olivocochlear bundle: Electrical... - Google Scholar*, Brain research.

Oertel, D. & Wu, S.H., 1989. Morphology and physiology of cells in slice preparations of the dorsal cochlear nucleus of mice - Oertel - 2004 - Journal of Comparative Neurology - Wiley Online Library. *Journal of Comparative Neurology*.

Oertel, D. & Young, E.D., 2004. What's a cerebellar circuit doing in the auditory system? *Trends in Neurosciences*, 27(2), pp.104–110.

Ohlrogge, M., Doucet, J.R. & Ryugo, D.K., 2001. Projections of the pontine nuclei to the cochlear nucleus in rats. *The Journal of Comparative Neurology*, 436(3), pp.290–303.

Osen, K.K., 1969a. Cytoarchitecture of the cochlear nuclei in the cat. *The Journal of Comparative Neurology*, 136(4), pp.453–484.

Osen, K.K., 1972. Projection of the cochlear nuclei on the inferior colliculus in the cat. *The Journal of Comparative Neurology*, 144(3), pp.355–372.

Osen, K.K., 1969b. The Intrinsic Organization of the Cochlear Nuclei in the Cat. *Acta Oto-Laryngologica*, 67(2-6), pp.352–359.

Parham, K. & Kim, D.O., 1995. Spontaneous and sound-evoked discharge characteristics of complex-spiking neurons in the dorsal cochlear nucleus of the unanesthetized decerebrate cat. *Journal of Neurophysiology*, 73(2), pp.550–561.

Parham, K. et al., 2000. Purkinje cell degeneration and control mice: responses of single units in the dorsal cochlear nucleus and the acoustic startle response. *Hearing research*.

Portfors, C.V. & Roberts, P.D., 2007. Temporal and Frequency Characteristics of Cartwheel Cells in the Dorsal Cochlear Nucleus of the Awake Mouse. *Journal of Neurophysiology*, 98(2), pp.744–756.

Poulet, J.F. & Hedwig, B., 2001. Tympanic membrane oscillations and auditory receptor activity in the stridulating cricket Gryllus bimaculatus. *J Exp Biol*, 204(Pt 7), pp.1281–1293.

Poulet, J.F.A. & Hedwig, B., 2003. A corollary discharge mechanism modulates central auditory processing in singing crickets. *Journal of Neurophysiology*, 89(3), pp.1528–1540.

Poulet, J.F.A. & Hedwig, B., 2007. New insights into corollary discharges mediated by identified neural pathways. *Trends in Neurosciences*, 30(1), pp.14–21.

Poulet, J.F.A. & Hedwig, B., 2006. The cellular basis of a corollary discharge. *Science*, 311(5760), pp.518–522.

Powell, E.W. & Hatton, J.B., 1969. Projections of the inferior colliculus in cat. *The Journal of*

Comparative Neurology, 136(2), pp.183–192.

Prosser, C.L. & Hunter, W.S., 1936. The extinction of startle responses and spinal reflexes in the white rat. *American Journal of Physiology-- …*.

Reiss, L.A.J., 2005. Spectral Edge Sensitivity in Neural Circuits of the Dorsal Cochlear Nucleus. *Journal of Neuroscience*, 25(14), pp.3680–3691.

Requarth, T. & Sawtell, N.B., 2014. Plastic corollary discharge predicts sensory consequences of movements in a cerebellum-like circuit. *Neuron*, 82(4), pp.896–907.

Requarth, T., Kaifosh, P. & Sawtell, N.B., 2014. A role for mixed corollary discharge and proprioceptive signals in predicting the sensory consequences of movements. *Journal of Neuroscience*, 34(48), pp.16103–16116.

Rhode, W.S., 1999. Vertical cell responses to sound in cat dorsal cochlear nucleus. *Journal of Neurophysiology*, 82(2), pp.1019–1032.

Rice, J.J. et al., 1992. Pinna-based spectral cues for sound localization in cat. *Hearing research*.

Ro, T. et al., 2009. Sound enhances touch perception. *Experimental Brain Research*, 195(1), pp.135–143.

Roberts, B.L. & Russell, I.J., 1972. The activity of lateral line efferent neurones in stationary and swimming dogfish

. *J Exp Biol*.

Roberts, P.D. & Bell, C.C., 2000. Computational consequences of temporally asymmetric learning rules: II. Sensory image cancellation. *Journal of Computational Neuroscience*, 9(1), pp.67–83.

Romfh, J.H., Capra, N.F. & Gatipon, G.B., 1979. Trigeminal nerve and temporomandibular joint of the cat: a horseradish peroxidase study. *Experimental neurology*, 65(1), pp.99–106.

Rose, J.E., Galambos, R. & Hughes, J.R., 1959. Microelectrode studies of the cochlear nuclei of the cat. *Bulletin of the Johns Hopkins Hospital*, 104(5), pp.211–251.

Roth, G.L. et al., 1978. Some features of the spatial organization of the central nucleus of the inferior colliculus of the cat. *The Journal of Comparative Neurology*, 182(4), pp.661–680.

Roy, J.E. & Cullen, K.E., 2004. Dissociating Self-Generated from Passively Applied Head Motion: Neural Mechanisms in the Vestibular Nuclei. *Journal of Neuroscience*, 24(9), pp.2102–2111.

Roy, J.E. & Cullen, K.E., 2001. Selective Processing of Vestibular Reafference during Self-Generated Head Motion. *The Journal of Neuroscience*.

Ryugo, D. & Willard, F.H., 1985. The dorsal cochlear nucleus of the mouse: a light microscopic analysis of neurons that project to the inferior colliculus. *The Journal of Comparative …*.

Ryugo, D.K., Willard, F.H. & Fekete, D.M., 1981. Differential afferent projections to the inferior

colliculus from the cochlear nucleus in the albino mouse. *Brain Research*, 210(1-2), pp.342–349.

Saade, N.E. et al., 1989. Dorsal column input to cochlear neurons in decerebrate-decerebellate cats. *Brain Research*, 486(2), pp.399–402.

Sawtell, N.B., 2010. Multimodal integration in granule cells as a basis for associative plasticity and sensory prediction in a cerebellum-like circuit. *Neuron*, 66(4), pp.573–584.

Sawtell, N.B. & Williams, A., 2008. Transformations of Electrosensory Encoding Associated with an Adaptive Filter. *Journal of Neuroscience*, 28(7), pp.1598–1612.

Schneider, D.M., Nelson, A. & Mooney, R., 2014. A synaptic and circuit basis for corollary discharge in the auditory cortex. *Nature*, 513(7517), pp.189–194.

Shore, S.E., 2005a. Multisensory integration in the dorsal cochlear nucleus: unit responses to acoustic and trigeminal ganglion stimulation - Shore - 2005 - European Journal of Neuroscience - Wiley Online Library. *European Journal of Neuroscience*.

Shore, S.E., 2005b. Multisensory integration in the dorsal cochlear nucleus: unit responses to acoustic and trigeminal ganglion stimulation. *European Journal of Neuroscience*, 21(12), pp.3334–3348.

Shore, S.E. & Zhou, J., 2006. Somatosensory influence on the cochlear nucleus and beyond. *Hearing research*, 216-217, pp.90–99.

Shore, S.E. et al., 2008. Dorsal cochlear nucleus responses to somatosensory stimulation are enhanced after noise-induced hearing loss. *European Journal of Neuroscience*, 27(1), pp.155–168.

Shore, S.E. et al., 2000. Trigeminal ganglion innervates the auditory brainstem. *The Journal of Comparative Neurology*, 419(3), pp.271–285.

Shore, S.E., Kashlan, El, H. & Lu, J., 2003. Effects of trigeminal ganglion stimulation on unit activity of ventral cochlear nucleus neurons. *NSC*, 119(4), pp.1085–1101.

Smith, M.A. & Shadmehr, R., 2005. Intact Ability to Learn Internal Models of Arm Dynamics in Huntington's Disease But Not Cerebellar Degeneration. *Journal of Neurophysiology*, 93(5), pp.2809–2821.

Sperry, R.W., 1950. Neural basis of the spontaneous optokinetic response produced by visual inversion. *Journal of Comparative and Physiological Psychology*, 43(6), pp.482–489. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-58149426727&partnerID=40&md5=49cfde87b468104385b63dd0aa0300f6.

Srivastava, C.B.L., 1977. Occurrence of sensory nerve-endings (stretch receptors) in the muscles of a teleost, Gnathonemus-Petersii. *Archives d'anatomie microscopique et de morphologie experimentale*, 66(4), pp.253–261.

Suemune, S. et al., 1992. Trigeminal nerve endings of lingual mucosa and musculature of the rat. *Brain Research*, 586(1), pp.162–165.

Sutherland, D., 1998. Role of acoustic striae in hearing: Reflexive responses to elevated sound-sources. *Behavioural brain research*, 97(1-2), pp.1–12.

Szabo, T., Libouban, S. & Denizot, J.P., 1990. A well defined spinocerebellar system in the weakly electric teleost fish Gnathonemus petersii. A tracing and immuno-histochemical study. *Archives italiennes de biologie*, 128(2-4), pp.229–247.

Takemura, M., Sugimoto, T. & Shigenaga, Y., 1991. Difference in central projection of primary afferents innervating facial and intraoral structures in the rat. *Experimental neurology*, 111(3), pp.324–331.

Tzounopoulos, T. et al., 2004. Cell-specific, spike timing–dependent plasticities in the dorsal cochlear nucleus. *Nature Neuroscience*, 7(7), pp.719–725.

Tzounopoulos, T. et al., 2007. Coactivation of pre- and postsynaptic signaling mechanisms determines cell-specific spike-timing-dependent plasticity. *Neuron*, 54(2), pp.291–301.

Van Noort, J., 1969. *The structure and connections of the inferior... - Google Scholar*,

Varecka, L. et al., 1994. GABAA/benzodiazepine receptor alpha 6 subunit mRNA in granule cells of the cerebellar cortex and cochlear nuclei: expression in developing and mutant mice. *The Journal of Comparative Neurology*, 339(3), pp.341–352.

Voigt, H.F. & Young, E.D., 1990. Cross-correlation analysis of inhibitory interactions in dorsal cochlear nucleus. *Journal of Neurophysiology*, 64(5), pp.1590–1610.

Voigt, H.F. & Young, E.D., 1980. Evidence of inhibitory interactions between neurons in dorsal cochlear nucleus. *Journal of Neurophysiology*, 44(1), pp.76–96.

Warr, W.B., 1966. Fiber degeneration following lesions in the anterior ventral cochlear nucleus of the cat. *Experimental neurology*, 14(4), pp.453–474.

Webster, D.B. & Trune, D.R., 1982. Cochlear nuclear complex of mice - Webster - 2005 - American Journal of Anatomy - Wiley Online Library. *American Journal of Anatomy*.

Weedman, D., 1996. Projections from auditory cortex to the cochlear nucleus in rats: synapses on granule cell dendrites. *The Journal of Comparative Neurology*.

Weinberg, R.J. & Rustioni, A., 1987. A cuneocochlear pathway in the rat. *NSC*, 20(1), pp.209–219.

Wickesberg, R.E. & Oertel, D., 1988. Tonotopic projection from the dorsal to the anteroventral cochlear nucleus of mice. *The Journal of Comparative Neurology*, 268(3), pp.389–399.

Wigderson, E., Nelken, I. & Yarom, Y., 2016. Early multisensory integration of self and source motion in the auditory system. *Proceedings of the National Academy of Sciences*.

Wouterlood, F.G. & Mugnaini, E., 1984. Cartwheel neurons of the dorsal cochlear nucleus: a Golgi-electron microscopic study in rat. *The Journal of Comparative Neurology*, 227(1), pp.136–157.

Wouterlood, F.G. et al., 1984. Stellate neurons in rat dorsal cochlear nucleus studied with

combined Golgi impregnation and electron microscopy: synaptic connections and mutual coupling by gap junctions - Springer. *Journal of neurocytology*.

Young, E.D., 1980. Identification of response properties of ascending axons from dorsal cochlear nucleus. *Brain Research*, 200(1), pp.23–37.

Young, E.D. & Brownell, W.E., 1976. Responses to tones and noise of single cells in dorsal cochlear nucleus of unanesthetized cats. *Journal of Neurophysiology*, 39(2), pp.282–300.

Young, E.D. & Voigt, H.F., 1981. The Internal Organization of the Dorsal Cochlear Nucleus. In *Neuronal mechanisms of hearing*. Boston, MA: Springer US, pp. 127–133.

Young, E.D., Nelken, I. & Conley, R.A., 1995. Somatosensory effects on neurons in dorsal cochlear nucleus. *Journal of Neurophysiology*, 73(2), pp.743–765. Available at: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=7760132&retmode=ref&cmd=prlinks.

Young, E.D., Rice, J.J. & Tong, S.C., 1996. Effects of pinna position on head-related transfer functions in the cat. *The Journal of the Acoustical Society of America*, 99(5), pp.3064–3076. Available at: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=8642117&retmode=ref&cmd=prlinks.

Zettel, M.L. et al., 2001. Early bilateral deafening prevents calretinin up-regulation in the dorsal cortex of the inferior colliculus of aged CBA/CaJ mice. *Hearing research*, 158(1–2), pp.131–138.

Zhan, X. & Pongstaporn, T., 2006. Projections of the second cervical dorsal root ganglion to the cochlear nucleus in rats - Zhan - 2006 - Journal of Comparative Neurology - Wiley Online Library. *Journal of Comparative …*.

Zhan, X. & Ryugo, D.K., 2007. Projections of the lateral reticular nucleus to the cochlear nucleus in rats. *The Journal of Comparative Neurology*, 504(5), pp.583–598.

Zhang, S. & Oertel, D., 1993. Cartwheel and superficial stellate cells of the dorsal cochlear nucleus of mice: intracellular recordings in slices. *Journal of Neurophysiology*, 69(5), pp.1384–1397.

Zhao, Y. & Tzounopoulos, T., 2011. Physiological Activation of Cholinergic Inputs Controls Associative Synaptic Plasticity via Modulation of Endocannabinoid Signaling. *Journal of Neuroscience*, 31(9), pp.3158–3168.

Zhou, J. & Shore, S., 2004. Projections from the trigeminal nuclear complex to the cochlear nuclei: A retrograde and anterograde tracing study in the guinea pig. *Journal of Neuroscience Research*, 78(6), pp.901–907.

Zuo, J. et al., 1997. Neurodegeneration in Lurcher mice caused by mutation in δ2 glutamate receptor gene. *Nature*.

Appendix A: Cartwheel cell responses to licking and mimic in hearing mice

Results in Chapter 3 showed that cartwheel cells in DCN of deafened mice responded to licking behavior. I also performed experiments observing cartwheel cell responses in the DCN of hearing mice to both licking and the mimic analogous to those experiments performed in Chapter 1. Since primary auditory afferents do not synapse onto cartwheel cells, cartwheel cell response to sounds would be expected to be weak. Indeed, previous studies in DCN have suggested that cartwheel cell respond weakly to noise and slightly stronger to pure tones (Parham & D. O. Kim 1995; K. A. Davis et al. 1996) including in the DCN of mice (Portfors & P. D. Roberts 2007; Ma & Brenowitz 2012). I therefore hypothesized that cartwheel cell responses to licking would be strong while their responses to the mimic would be weak.

Interestingly, this was not the case. Both complex spikes and simple spikes of cartwheel cells showed significant modulations to both licking and the mimic (**Figure 1a**). Z-scored complex spike responses to the lick were not significantly different from z-scored responses to the mimic (n=8, P=0.64, Wilcoxon Sign Rank Test). Similarly z-scored simple spike responses to the lick were not significantly different from z-scored responses to the mimic (n=8, P=.11, Wilcoxon Sign Rank Test). However, while not significant, there is a definite trend towards higher complex spike rates while licking versus with the mimic (n=8; 2.10 Hz ± 1.73 Hz for mimic; 3.57 Hz ± 1.97 Hz for licking, mean and standard deviation; P=0.054, Wilcoxon Sign Rank Test, **Figure 1b**). This was

not the case for simple spikes (n=8; 25.58 Hz ± 9.22Hz for mimic; 24.8 Hz ± 12.47 Hz for licking; P=.25, Wilcoxon Sign Rank Test). This suggests that auditory and non-auditory inputs play an important role in cartwheel cell function.

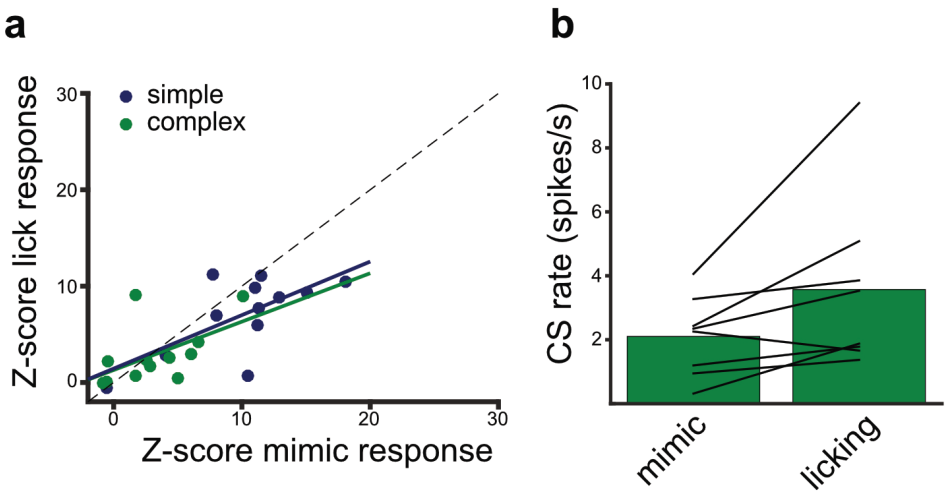

Figure 11 | Cartwheel cell responses to mimic and licking. **a,** Z-scored lick response plotted as a function of z-scored mimic responses in both simple (blue) and complex (green) cells. Complex and simple spikes of cartwheel cells responded to both the mimic and the lick. Dotted line represents unity line. **b,** Complex spike rates were larger when the mouse was licking than when the mimic was being played. Each line represents one unit while bars represent mean complex spike firing rate in each condition.